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Too narrow-minded about energy?

THE gilt text inscribed around the ceiling of Church House, Westminster, speaks of "true light", "lending radiance" and "enduring in the heat" (admittedly "of the conflict"). A highly appropriate setting for Mr Anthony Wedgwood Benn's National Energy Conference last week, to which 470 were invited and at which 56 spoke.

Let it first be said that all those reasonable doubts that such an event could take place without degenerating into a shambles turned out to be unjustified, for which great credit to Mr Benn's chairmanship (and not least his restraint in not intervening in the debate at all himself) and to the speakers who never once exceeded their time limit. But there is a world of difference between a well-ordered debate and open government; has Mr Benn done anything beyond ensure that all interested parties had a six-minute airing of the views?

There is no doubt the meeting was valuable in mutual education terms; almost all attenders had at least the courtesy to sit and listen to all contributions rather than break up into factions in the corridor to discuss heaven knows what. But what can Mr Benn and his staff do as a result of it all that he couldn't have done the day before?—for it can reasonably be assumed that he, as an exponent of open government, will have been familiar with all the points raised—even Sir Brian Flowers warning that the Royal Commission on Environmental Pollution will raise serious questions about living with plutonium, and even Mr Enoch Powell's brilliant oratory against any form of energy policy-making as ill-conceived and pernicious.

That Mr Benn and his department will have learnt little new from his conference is really the result of attempting to get too many things in focus in one day. The most incongruous of them was the question of disconnections of electricity and gas for non-payment. This was a bandwagon tailor-made for union leaders to jump on to, and although it provided some stirring talk, it managed to confuse a conference convened to talk about energy policy by diverting attention to matters of social policy, which should not be within Mr Benn's terms of reference.

Another structural weakness of the day was the lack of a challenging position paper. Before the conference everyone had been flooded with documents representing the positions of every conceivable interest, but the Department of Energy had, it seemed, decided to maintain a low profile. Its agenda and discussion paper was little more than a list of subject headings—and even that was largely ignored by speakers.

In hindsight, this was probably unfortunate. What was really needed was a major presentation, perhaps an hour in

length and certainly well illustrated (not a slide was shown all day!), skilfully aimed at raising the more provocative issues and setting a more definite tone for the day. Perhaps Mr Benn will try this, as he undoubtedly has plans for future, smaller gatherings.

But whatever the next move is that Mr Benn has up his sleeve, his eventual plans for the gas industry are likely to provide the most sensitive litmus of true commitment to "open government". When Lord Ryder stood up to dismiss as economically senseless the calls for a competitive restrictive tariff on North Sea gas, he was not only supporting the gas industry itself, but also voicing the majority opinion among institutionally disinterested energy experts. Lord Ryder's reference to a 1976 version of the mad hatter's tea party, and his warning of the dangers of a "wretched cartel with coal and electricity" will ensure one thing at least. If Mr Benn, often accused of a fondness for dogmatism, eventually opts for the recommendations of his political advisers and thus weighs down the nation's only economically sound energy industry, he will need a convincing case to defend himself against allegations of blind adherence to doctrine. While some sympathy must be felt for the coal and electricity chiefs, struggling through a difficult period, on current analyses a gas tax provides a poor solution to their own dilemma, and a poorer answer to the nation's broader, long-term energy problems.

The difficulties of the gas industry perhaps underline a disturbing thread running through the day (and conceivably the result of no strong position paper): the narrow-mindedness of most participants. An unspoken assumption seemed to be that self-sufficiency in Britain was the aim and if we talked about energy in any international forum at all (and almost all didn't) it was to be from a position of independence. This was the much more explicit objective, two years ago, of Project Independence in the United States. It assumes that in the long run no foreigners are trustworthy. There is much danger in such chauvinism, which may go down well in some political circles but which could lead to some ridiculously expensive decisions being made in pursuit of xenophobia.

Britain in general depends heavily on importing and exporting and is inevitably vulnerable to the capriciousness of suppliers of raw materials and food. But foreign policy, recognising no alternative to large-scale importing, is aimed at maintaining the appropriate good relations. Should energy be an exception to be protected by vast expenditures in research, development and implementation? This is one issue we would like to hear Mr Benn speak more of. □



Genetic manipulation: guidelines issued

The NIH ground rules for genetic manipulation experiments may not mark the end of an unprecedented debate within the scientific community. **Colin Norman** reports from Washington

AFTER two years of controversy and uncertainty, the National Institutes of Health (NIH) last week issued a complex set of guidelines governing the use of a powerful new technique for manipulating genes in living organisms. The guidelines establish safety rules for experiments which may revolutionize biology, but which also provide man with unprecedented ability to alter the characteristics of living things. They are, however, far from being the final word on whether, and under what circumstances, such research should be allowed to go ahead, for they are already being overtaken by events in some places.

On July 7, for example, the City Council in Cambridge, Mass., will vote on a resolution, proposed by the Mayor, which would ban for two years all such experiments at Harvard and MIT. And months of bitter debate at the University of Michigan have resulted in the adoption of regulations there which are more strict than those issued by NIH last week. The focus of the debate is clearly shifting from Washington into the university communities where the research will take place.

Nevertheless, the NIH guidelines will provide important ground rules for genetic manipulation experiments in many institutions, and their impact will extend far beyond the borders of the United States, for they are likely to influence the establishment of guidelines in many other countries. They have been developed by an extraordinary process of self-regulation by the scientific community.

The process began in 1973, when scientists familiar with the nascent technique began to worry about potential hazards associated with its use. Their concerns led a committee of the National Academy of Sciences to issue a public statement in July 1974, urging scientists around the world to defer two types of experiments until the hazards have been defined. The moratorium lasted until last February, when it was partly replaced by general guidelines recommended by an international group of geneticists which met at Asilomar, California. Then an NIH advisory committee, consisting of scientists, took centre stage. It laboured hard during most of last year trying to cast the Asilomar guidelines into more specific rules, completing its task by hammering out a set of complex proposals last December. The proposals went to NIH Director Donald S. Fredrickson, who

called a public meeting to discuss them, solicited the views of numerous scientists and non-scientists, and asked the advisory committee to reconsider some of its suggestions. The guidelines issued last week represent Fredrickson's distillation of the conflicting advice presented to him.

They differ a little in detail, but not in philosophy, from the recommendations of the advisory committee. They will allow most planned experiments to go ahead, albeit under strict safety controls, outlawing only a handful of the more hazardous types of experiments. They are, however, stricter than the Asilomar guidelines, a fact which Fredrickson suggested means that "the research will go forward in a manner responsive and appropriate to hazards that may be realised in the future". And Dr DeWitt Stetten, Deputy NIH Director for Science and chairman of the advisory committee, argued last week that "the issuance of the guidelines is in no sense an opening of the floodgates, rather it is a closing of the leaks (in the Asilomar guidelines)".

Be that as it may, the guidelines are not even NIH's final say on the matter. Their publication falls under the terms of the National Environmental Policy Act, which means that NIH must prepare an assessment of the potential impact of the research on the environment. A draft assessment should be ready by September, and since it will be opened up for public comments before being cast in final form, another round of discussion of the risks and benefits of the research is in store. In the meantime, the guidelines will take effect, governing NIH's support of the research.

New dimension

Why have the guidelines taken so long to produce, and caused so much strife? The answer is that the research offers a spectacular mix of potential benefits and possible hazards, and it opens up an entirely new dimension in biology. The experiments consist, in short, of snipping genes from the DNA of any organism and splicing them into the DNA of another, perhaps entirely unrelated, organism. The resulting molecule—a recombinant DNA molecule—is copied (cloned) each time the new host reproduces, producing large quantities of the transplanted genes. The technique offers a powerful tool for probing the working of genes and their arrangement in complex organisms and,

more distantly, it may offer a means of constructing special micro-organisms for a variety of medical, commercial and industrial uses.

But the worry is that the technique allows biologists to breach genetic barriers between species which have evolved over thousands of years. In short, it allows biologists to by-pass the processes of evolution. More specifically, foreign genes inserted into an organism may cause that organism to behave in a dangerous, and perhaps unpredictable, manner. The guidelines thus seek to ensure that micro-organisms bearing transplanted genes are contained in the laboratory.

They spell out our levels of physical containment to be used in such experiments, designated P1 to P4, ranging from use of standard microbiological techniques (P1) to the use of specially equipped facilities akin to biological warfare laboratories (P4). And, as a second line of defence, they spell out three levels of biological containment, EK1 to EK3, which must be used for experiments involving the insertion of genes into a strain of the common gut bacterium *E. coli*—the organism which will be used for most experiments. The levels are as follows:

●EK1—use of standard *E. coli* K12, a laboratory strain of *E. coli* which has been used for genetic experiments for decades. Foreign genes are inserted into the bacterium by splicing them into a plasmid (a ring of bacterial DNA which reproduces independently from the bacterium's chromosomes) and reintroducing the recombinant into the bacterium, or by splicing them into the DNA of a bacteriophage which then infects *E. coli* K12.

●EK2—the use of specially mutated strains of *E. coli* or bacteriophage which, according to laboratory tests, are virtually incapable of surviving outside the laboratory so that the recombinant DNA will have less than 1 in 10^{-8} chances of surviving in the natural environment.

●EK3—the same as EK2 except that the survivability has been tested in animals, plants and other environments.

The guidelines assign specific physical and biological safety levels to various types of experiments on the basis of their potential hazards (see box). They

also spell out safety rules for experiments involving recombinants formed by splicing genes into the DNA of animal viruses, which are then grown in cell cultures to provide multiple copies of the foreign genes. The final guidelines alter some of the containment levels proposed by the advisory committee, but no substantial alterations have been made.

Cautious approach

How did Fredrickson arrive at his final decision when confronted with such a wealth of conflicting advice? His first, and most fundamental, consideration was simply whether the research should be allowed to go ahead at all, in view of the potential hazards. The majority of the commentators on the guidelines recommended that it should, but he was advised to take an extremely cautious approach by two of the most eminent of the commentators, neither of whom intends to conduct experiments with recombinant DNA.

Dr Robert Sinsheimer, chairman of the Department of Biology at California Institute of Technology, argued in a letter to Fredrickson that the experiments present a serious hazard if they breach the genetic barrier between higher organisms (eukaryotes) and lower organisms (prokaryotes). "One need not continue to spin out potential horror stories", he wrote, "the point is that we will be perturbing, in a major way, an extremely intricate ecological interaction which we understand only dimly". As for the guidelines themselves, Sinsheimer stated: "I cannot believe that under these proposed guidelines the organism can be contained. If the work is going on in a hundred laboratories about the United States, performed by technicians, graduate students, etc., the organism will inevitably escape—and will enter into the various ecological niches known to be inhabited by *E. coli*." He therefore proposed that all work with recombinant DNA should be performed under maximum containment conditions at a single institution in the United States, and that there should be an intensive effort to seek a micro-organism more suitable than *E. coli* for the work.

The other eminent, though more flamboyant, critic of the guidelines, Erwin Chargaff of Columbia University, recommended that all work on recombinant DNA should be halted for at least two years to allow time for the hazards to be assessed. Chargaff asked, in a letter published in *Science*, "Have we the right to counteract, irreversibly, the evolutionary wisdom of millions of years, in order to satisfy the ambition and the curiosity of a few scientists?"

The advisory committee did not consider Sinsheimer's or Chargaff's pro-

Guidelines in detail

The guidelines define four levels of physical containment, designated, in order of increasing stringency, P1 to P4, and three levels of biological containment, EK1 to EK3, and assign experiments to them on the basis of potential risk. The following is a summary of containment levels specified for various sources of DNA.

a. Shotgun experiments using *E. coli* as the host

Non-embryonic primate tissue	P3 + EK3 or P4 + EK2
Embryonic primate tissue or germ line cells	P3 + EK2
Other mammals	P3 + EK2
Birds	P3 + EK2
Cold blooded vertebrates, non-embryonic	P2 + EK2
embryonic or germ line	P2 + EK1
If vertebrate produces a toxin	P3 + EK2
Other cold blooded animals and lower eukaryotes	P2 + EK1
If Class 2 pathogen*, produces a toxin, or carries a pathogen	P3 + EK2
Plants	P2 + EK1
Prokaryotes that exchange genes with <i>E. coli</i>	
Class 1 agents (non-pathogens)	P1 + EK1
Low risk pathogens (for example, enterobacteria)	P2 + EK1
Moderate risk pathogens (for example, <i>S. typhi</i>)	P2 + EK2
Higher risk pathogens	banned
Prokaryotes that do not exchange genes with <i>E. coli</i>	
Class 1 agents	P2 + EK2 or P3 + EK1
Class 2 agents (moderate risk pathogens)	P3 + EK2
Higher pathogens	banned

In all above cases, if DNA is at least 99% pure before cloning and contains no harmful genes, either physical or biological containment levels can be reduced one step.

b. Cloning plasmid, bacteriophage and other virus genes in *E. coli*

Animal viruses	P4 + EK2 or P3 + EK3
If clones free from harmful regions	P3 + EK2
Plant viruses	P3 + EK1 or P2 + EK2
99% pure organelle DNA, Primates	P3 + EK1 or P2 + EK2
other eukaryotes	P2 + EK1
Impure organelle DNA: shotgun conditions apply.	
Plasmid or phage DNA from hosts that exchange genes with <i>E. coli</i>	
If plasmid or phage genome does not contain harmful genes or if DNA segment 99% pure and characterised	P1 + EK1
Otherwise, shotgun conditions apply.	

Plasmids and phage from hosts which do not exchange genes with <i>E. coli</i>	
Shotgun conditions apply, unless minimal risk that recombinant will increase pathogenicity or ecological potential of the host, then	P2 + EK2 or P3 + EK1

NB. cDNAs synthesised *in vitro* from cellular or viral RNAs are included in above categories.

c. Animal virus vectors

Defective polyoma virus + DNA from non-pathogen	P3
Defective polyoma virus + DNA from Class 2 agent	P4
If cloned recombinant contains no harmful genes and host range of polyoma unaltered, reduce to	P3
Defective SV40 + DNA from non-pathogens	P4
If inserted DNA is 99% pure segment of prokaryotic DNA lacking toxigenic genes, or a segment of eukaryotic DNA whose function has been established and which has previously been cloned in a prokaryotic host-vector system, and if infectivity of SV40 in human cells unaltered	P3
Defective SV40 lacking substantial section of the late region + DNA from non-pathogens, if no helper used and no virus particles produced	P3
Defective SV40 + DNA from non-pathogen can be used to transform established lines of non-permissive cells under P3 provided no infectious particles produced. Rescue of SV40 from such cells requires	P4

d. Plant host-vector systems

- P2 conditions can be approximated by insect-free greenhouses, sterilization of plant, pots, soil and runoff water, and use of standard microbiological practice.
- P3 conditions require use of growth chambers under negative pressure and routine fumigation for insect control.
- Otherwise, similar conditions to those prescribed for animal systems apply.

*Classes for pathogenic agents as defined by the Center for Disease Control.

posals during its public meetings, but Fredrickson alluded to them in a lengthy statement published along with the guidelines. Recognising that the breaching of genetic barriers might pose a hazard, Fredrickson nevertheless argued that the research can be controlled so that it is carried out safely. Noting that "the international scientific community . . . has indicated a desire to proceed with research in a conservative manner", and that "most of the considerable public commentary on the subject, while urging caution, has also favoured proceeding", Fredrickson pointed out that there is, in any case, no way to prohibit the research throughout the world. "There is," he added, "no reason to attempt it."

Having decided that there should be no flat proscription on the research, Fredrickson turned to some of the chief concerns raised by the critics of the guidelines. The most prominent concern arises from the fact that most work with recombinant DNA will take place with the *E. coli* K12 bacterium. Since *E. coli* is a common inhabitant of the human gut, many observers have considered it a dangerous choice for the research. In particular, a group of scientists from the Boston area urged that a different host for transplanted genes be developed, and that the use of *E. coli* be phased out as swiftly as possible.

Fredrickson argues, however, that since the bacterium has been used for decades as the geneticists' workhorse, there is extensive knowledge of its behaviour and, moreover, there is good evidence that it is unlikely to survive for long in the environment in competition with wild strains of *E. coli*. In other words, *E. coli* K12 itself provides a level of biological containment. "I believe that because of this experience, *E. coli* K12 will provide a host-vector system that is safer than other systems", Fredrickson argued, and he declined to set a limit on when it should be phased out of the research.

Another area of concern with the proposed guidelines centred on how they should be implemented, and that section has been extensively revised. The guidelines strictly apply only to research supported by NIH, and investigators must comply with them before they can receive a grant. Some commentators suggested that principal investigators be required to obtain informed consent from all laboratory personnel before proceeding with recombinant DNA experiments, but Fredrickson opted instead for a requirement that the investigators simply inform all laboratory workers of the real and potential hazards associated with the experiments.

The guidelines also require that each

institution where recombinant DNA experiments will be conducted should establish a biohazards committee, to ensure that facilities meet specified requirements, training is adequate and so on. The advisory committee had firmly recommended that such committees should not be responsible for determining the containment conditions for specific experiments, but Fredrickson deleted that prohibition, leaving the matter up to individual institutions.

A final point concerning implementation which is clearly worrying many people is that the guidelines do not apply to industry. Early last month, however, Fredrickson briefed officials from various industries on the guidelines and received from them expressions of support for their intent and their general provisions. Some officials expressed reservations about specific items, however, such as the provision prohibiting large-scale experiments with recombinant DNA, and the Pharmaceutical Manufacturers' Association has decided to convene a committee to review whether the guidelines are applicable to the drug industry.

Effect of guidelines

Now that these guidelines have been issued, how do they affect academic scientists conducting, or hoping to conduct, recombinant DNA experiments? First, they specify that many experiments should use EK2 or EK3 biological containment, and until crippled micro-organisms which meet those criteria are available, such experiments should not be conducted. The advisory committee which drafted the guidelines is responsible for certifying whether crippled strains meet the criteria.

A strain of *E. coli* produced by Roy Curtiss of the University of Alabama (called $\psi 1776$) has been proposed as an EK2 system with two specific plasmids (pSC101 and Col El-kan). Curtiss, who has built many crippling mutations into the bacteria in an effort which took the best part of a year, has provided the committee with reams of data on its survivability. Last month, a subcommittee voted unanimously that the strain meets the EK2 specifications, and the full committee is expected to approve it in the next few weeks. That would pave the way for many experiments.

But a confusing situation has developed with respect to two other candidate EK2 strains. At its last meeting in April, the committee approved a strain of bacteriophage lambda, developed by Philip Leder of NIH as an EK2 strain. But a subcommittee which met last month to consider a second candidate strain, developed by Fred Blattner at the University of Wisconsin, was divided on a matter which

affects both strains. In short, two subcommittee members refused to endorse the use of either phage as an EK2 system unless they are used in conjunction with crippled bacteria. The full committee will take up that thorny issue when it next meets in September. Curtiss's *E. coli* strain, incidentally, is resistant to infection by phage λ .

Many of the objections and reservations which critics have levelled at the guidelines are now beginning to crop up in local debates in and around the universities where the research will be conducted. In that regard, the situation brewing in Cambridge, Mass., may be an indication of things to come. It began quietly a few months ago, when some researchers at Harvard proposed that a laboratory, meeting P3 requirements, be established in the Harvard Biology Labs. The facility, which would cost some \$350,000, would have involved converting some existing laboratory space, and the intent was to undertake a variety of work in it, including some recombinant DNA experiments.

The proposal met with opposition within Harvard, however, largely because the biology labs are old, infested with cockroaches and with a species of ant which has so far evaded attempts at eradication. In short, critics suggested that the building is unsuitable for a P3 facility. Their criticisms broadened into an assault on the proposal to conduct recombinant DNA experiments at Harvard, however, and the dispute spilled over into the city when the matter was reported at length in Boston's weekly newspaper.

Approval of the laboratory by the Harvard authorities was all but assured, since the concept had been endorsed by a biohazards committee, Harvard's Committee on Research Policy, and the Dean of Arts and Sciences. But, when he read about the dispute in the newspapers, Cambridge city Mayor Alfred Velucci stepped in. He called a council meeting on June 23—ironically the day the NIH guidelines were issued—to discuss the matter, and a string of scientists testified about the potential hazards and benefits of recombinant DNA research. By all accounts, the atmosphere was highly charged, and there was considerable heated discussion.

The matter has now gone well beyond the issue of whether the laboratory should be built, however, for Velucci has introduced a resolution which would prohibit all recombinant DNA experiments in Cambridge—even those deemed to have minimal risk—for two years. The council will vote on the resolution on July 7, and the outcome will be closely watched around the country. □

news and views

Prediction of volcanic activity and climate

from P. M. Kelly and H. H. Lamb

CURRENT interest in climatic change has stemmed from the growing awareness that man's activities are everywhere circumscribed by his environment, and in particular, by the vagaries of climate. The fluctuations of weather and climate in most parts of the world have been increasingly troublesome in the past 20–25 yr. This has happened partly because of an apparently real increase in the variability from year to year, or from one group of about 5 yr to the next, which has affected the Indian monsoons and temperatures and rainfalls in middle latitudes of both hemispheres, and partly because the growth of population and the rationalisation of agriculture towards specialisation in one-crop economies in various regions has increased vulnerability to weather. Climatic change has provided a control on human affairs in certain regions in the past and now, with the ever increasing economic interdependence of nations, its effects must be considered in future planning in all spheres—industrial, social and economic. Unfortunately this need for climatic forecasts cannot yet be adequately catered for by meteorologists or climatologists, who are still trying to determine the causes of climatic change by study of the limited climatic record.

In recent years much evidence, both theoretical and observational, has been put forward suggesting that the amount of volcanic dust in the upper atmosphere may be the major control of climatic change during the postglacial. Stratospheric dust effectively reduces the amount of solar energy reaching the Earth's surface where this energy is made available to the atmospheric circulation and variations in the amount of this pollutant, caused by the varying frequency and magnitude of explosive volcanic eruptions, may produce corresponding fluctuations in the

nature of the atmospheric circulation and climate. If this hypothesis is correct, prediction of climate will first necessitate prediction of volcanic activity. Although great steps are being taken in the forecasting of volcanic activity on time scales of days, prediction of volcanic activity on time scales of years and decades has been considered a remote possibility (and perhaps considered by many a distinct impossibility).

In last week's issue of *Nature*, Roosen *et al.* (261, 680; 1976) attempted to determine one cause of fluctuations in volcanic activity on time scales of centuries and provided a possible tool for the forecasting of tectonic activity and climate. They postulate that changes in the degree of tectonic activity may arise from variations in the rate of change of lunar tidal stress effects on the Earth's core and crust. Calculation of the long term variation of the tidal stress reveals a cycle of about 179 yr due to resonance effects arising from shorter period variations in the lunar orbit. There are many references to a cycle of about this length in the literature concerning climatic change, although in most cases the length of record analysed has been too short to evaluate the statistical significance and precise period of this fluctuation; its existence is not well-established. Spectral analysis of the Camp Century, Greenland ice core δO^{18} record reveals a cycle of about 180 yr and Roosen *et al.* claim that 13% of the variance of the δO^{18} record can be explained by the derivative of the tidal stress curve during the period AD1200 to AD1970. The correlation breaks down during the periods AD1420 to AD1530 and AD1650 to AD1700, an effect which the authors tentatively attribute to dominance of the effect of the exceptionally low degree of solar disturbance during those spans of

years.

Roosen *et al.* note that a cycle of about 180 yr has been observed in variations in solar activity, thereby providing an alternative explanation for this variation in climate. Therefore, before the climatic cycle of 180 yr can be used for forecasting purposes, it is essential that its existence be confirmed by analysis of other long climatic records and that its cause, solar and/or tectonic, be defined.

Research related to the past record of climate and the apparent causes, and rapidity, of climatic fluctuations and changes is being undertaken by scientists in many fields other than climatology. This is providing a great stimulus with many opportunities of increasing knowledge and verifying diagnoses as new data and new techniques of measurement and analysis are put forward. It is important that collaboration between workers in all the fields involved in the study of climatic change be initiated and fostered. A multidisciplinary approach is needed, both to compile the evidence of the facts of the past climatic record and to solve the problems of the causes of climatic change, which seem to extend from the Earth's core to the Sun (and, on the longest time scales, to the Galaxy beyond). Only from the work of organisations, ranging from the powerfully equipped mathematical laboratories of the atmospheric, oceanographic and Earth sciences to academic institutes and meetings where diverse specialists can be brought together, will the knowledge and understanding needed to develop sound advice on probable future climatic development be attained. The specialists will include agriculturists and forestry research workers, palaeobotanists, geologists and, in some cases, archaeologists and interpreters of ancient texts and inscriptions. □

Activation analysis *in vivo*

from Keith Boddy

The Second East Kilbride Conference on Progress and Problems of *In vivo* Activation Analysis, organised by the Scottish Universities Research and Reactor Centre, was held on April 6-9, 1976.

DELIBERATE induction of radioactivity in animal tissues was reported in *Nature* almost 27 yr ago and some 12 yr have passed since the feasibility of *in vivo* neutron activation analysis in man was demonstrated. The technique involves exposure of the total body or the relevant region of the body, such as the thyroid, liver, spine or limb, to a low dose of neutrons and measurement of either the induced radioactivity or the prompt gamma rays emitted during irradiation. Total body *in vivo* neutron activation analysis (TBIVNAA) is a unique method providing direct and simultaneous measurements of the total body content of calcium, phosphorus, nitrogen, sodium and chlorine. Similarly, there are partial body methods (PBIVNAA) for determining the total thyroid content of iodine, calcium and phosphorus in sections of bone or cadmium and copper in the liver. The reproducibility of measurements is generally 2-4%, permitting the detection of relatively small changes in body composition due to disease or treatment, the patient being examined on an out-patient basis.

It was clear from the increasing number of centres using, or proposing to use, *in vivo* activation analysis and from the wide range of clinical applications described at the conference that the potential of the technique in medicine is being realised. Several groups are using TBIVNAA in examinations of osteoporosis, osteomalacia, renal failure, hyperparathyroidism and endocrine dysfunctions. Regional measurements of calcium in bone are being made in some of these disorders and of thyroid iodine in hyperthyroidism.

The facilities at Brookhaven National Laboratory, New York, comprising 14 sources each of 50 Ci Pu Be distributed along the anterior and posterior body surfaces for irradiation and the sophisticated whole-body counter using 54 sodium iodide detectors, were described by S. H. Cohn. A wide variety of clinical studies are in progress: in thalassaemia, for example, gross depletion of skeletal calcium (50%) has been measured.

J. H. Fremlin (University of Birmingham) and his colleagues use a cyclotron for clinical measurements involving delayed gamma-ray emission and also for prompt gamma-ray analysis of total body nitrogen using hydrogen as an internal standard, as well as for the detection of cadmium in liver. In regional analyses, spinal calcium is measured. The feasibility of determining copper and iron in liver in pathological situations has been demonstrated using PBIVNAA, and preliminary studies of their measurement by nuclear resonance fluorescence were described.

W. B. Nelp (University of Washington) and H. E. Palmer (Battelle Northwest Laboratories, Washington) and coworkers also use a cyclotron in a wide range of clinical studies. The technique of measuring total body calcium using the reaction $^{40}\text{Ca}(n,\alpha)^{37}\text{Ar}$, first demonstrated by Palmer, in which the exhaled gas is collected, purified and counted in proportional counters, is now in clinical use. To measure calcium alone, smaller radiation doses can be used and whole-body counting is not required. This technique has also been adopted at Birmingham and at the Memorial Sloan Kettering Cancer Center, New York (J. S. Laughlin). The rate and completeness of exhalation of ^{37}Ar has been examined in detail by several groups. Although the exhalation pattern found was obviously complex, there was preliminary evidence that the technique was reproducible and could estimate total body calcium quantitatively in man.

The Medical Research Council cyclotron at Hammersmith Hospital is used by T. Spinks and colleagues for similar studies, and in some cases they measure calcium in the hand and the rate of removal of induced ^{24}Na .

In the system used at the University of Toronto by K. G. McNeil and J. E. Harrison, 12 sources each of 5 Ci of Pu Be are distributed only about the thorax and lumbar region of the patient, precluding absolute measurements of total body content. But calcium and sodium indices have been derived, relating the expected counting-rates of these elements to body habitus, and the measured counting rate in the patient is compared with that expected as an indicator of normality. This approach has been used in various clinical disorders. Measurement of nitrogen *in vivo* is also being attempted by the prompt gamma-ray method.

At East Kilbride, K. Boddy's group uses scanning irradiation with two sealed-tube 14-MeV neutron generators and a scanning shadow-shield whole-body counter for TBIVNAA. Changes of body composition in rheumatoid arthritis during treatment with non-

steroid analgesics and with corticosteroids have been studied. The nuclear reactor is used to measure the thyroidal content of iodine in hyperthyroid patients, as M. J. Hooper (Western Infirmary, Glasgow) reported. A technique of "compound irradiation" with mixed neutron beams has been developed to improve the uniformity of activation in partial body calcium measurements.

A facility using two californium-252 neutron sources each of 200 μg for partial body *in vivo* neutron activation analysis, developed by the East Kilbride group, is being used for clinical studies by M. A. Smith and colleagues at the Western General Hospital, Edinburgh. A much larger californium-252 source of 3 mg is used by A. D. LeBlanc and colleagues at Baylor College and the NASA/Johnson Space Centre, Houston, in a facility for measuring regional changes in skeletal calcium in man and whole-body measurements of small animals. A facility described by B. Mazière (CEA, Orsay), uses two sources each of 200 μg of ^{252}Cf together with four sources each of 10 Ci of ^{238}Pu -Be for the simultaneous measurement of calcium and phosphorus in the hand.

Many participants felt it was desirable to be able to compare directly the results from different centres. A proposal by Palmer to establish an international intercomparison, based on anthropomorphic phantoms containing human skeletons, to be measured "blind" by the various groups and subsequently analysed chemically, was received enthusiastically. Palmer and Boddy were appointed co-chairmen with the task of establishing the inter-laboratory comparison. □

Influence of grazing

from Peter D. Moore

SOME controversy surrounds the degree of influence exerted by man's grazing animals on the vegetation of the arid parts of the world. There is much room for argument and speculation simply because there is a paucity of factual information, particularly regarding the history of vegetation development. The processes at work have taken considerable periods of time—many millennia in the case of the Middle East—and the separation of climatic and anthropogenic influences often proves impossible in attempts to assess the underlying factors which have determined the structure and composition of extant vegetation. Some effort has recently been made to approach the

problem from the point of view of excluding grazing animals and observing the development of plant communities within enclosures. But at best, such experiments will take many decades before any important conclusions can be drawn, and it is possible that some species will no longer be in a position to reinvade such experimental plots.

The problems faced in such places as North America and Australia are rather different from those of the Old World in that the history of man's pastoral activity is much shorter and is generally well documented. In such conditions there is a better chance of elucidating any changes in vegetation which have occurred during this time. One approach which has recently been used is the analysis of population age structure in selected arid shrubs. If grazing pressures have severely influenced seedling survival, it should be possible to demonstrate that the younger shrubs are present at lower densities than would be expected for the maintenance of overall population levels. Crisp and Lange (*Oikos* **27**, 86; 1976) describe just such a study which they have undertaken on the shrub *Acacia burkittii* at Koonamore Station, a sheep-grazing area of southern Australia.

Sheep grazing began at Koonamore in the 1860s at a density of 0.4 sheep ha⁻¹, and it is recorded that within 50 yr the vegetation had become badly degraded. In 1925 an area of this range (400 ha) was fenced to exclude rabbit and sheep grazing and studies of consequent vegetation change have been carried out. Some permanent plots were marked out and within these the *Acacia burkittii* has become established. As a result precise ages are known for several of these shrubs. On the basis of this knowledge, Crisp and Lange have produced a linear regression of a bush size index (height plus diameter) against age. Even in the absence of annual growth rings this permits the estimation of shrub age on the evidence of its size, though data are available only for shrubs less than 42 yr old. Similar calculations were made for a stem girth/age relationship and it was assumed that a linear extrapolation could be made from this to estimate the ages of the older shrubs (maximum calculated age was about 250 yr).

These figures can be used for the examination of the age structure of *Acacia* populations, and this has been done both in regions with almost a century of sheep grazing in their history and also in those which have experienced no sheep grazing, but only that of rabbits. Population histograms from all sites examined show high frequencies of shrubs of a century in age, but lower frequencies of younger

shrubs. In a stable situation we would expect younger individuals to be at least as frequent as older ones up to the development of senescence. Reduced frequency of juveniles can often be correlated with the commencement of sheep grazing in the latter part of the nineteenth century, though rabbit grazing presents a complicating factor. In one site where there has been no sheep grazing, but only rabbit grazing, there is still a reduced frequency of shrubs in the 25–50 yr age group. Where grazing has been maintained to the present, the occurrence of *Acacia* shrubs less than 50 yr old is very low, or such juveniles are absent. Where sheep grazing was curtailed in 1925, there is a high frequency of young individuals, in spite of continued rabbit grazing.

There are several possible explanations for the form of these histograms apart from the sheep grazing effect. It is possible that a change in a climatic factor about 100 yr ago reduced the survival potential of *Acacia burkittii*. Crisp and Lange claim that reproduction in this shrub is not significantly affected by rainfall, but it is possible that the population of a natural predator of the seeds or seedlings, vertebrate or invertebrate, may be so influenced. There is also a possibility that *Acacia burkittii* is not a climax adapted species, but occupies a temporal niche in a successional development. There are many instances on record of species which invade and may even dominate a habitat for several decades during the course of succession but which fail to maintain viable populations in competitive conditions. For example, birch (*Betula pendula*) in temperate European forests invades areas after catastrophes and produces even aged populations. Twenty or 30 yr after invasion, the age structure of the population would look very similar in its basic shape to that of *Acacia*, for seedling establishment under a birch canopy is very poor in this species. Some successional dominants, such as *Typha latifolia* (McNaughton, *Ecology*, **49**, 367; 1968) secrete compounds which directly inhibit seedling germination which has the advantage of reducing self-competition.

Such alternative explanations, however, do not explain how the removal of sheep grazing can so profoundly increase the recruitment of new seedlings into the *Acacia* populations. By far the simplest explanation is the one propounded by Crisp and Lange, that the introduction of sheep grazing in addition to natural predation and increasing rabbit predation, has placed an unacceptable demand on the regeneration potential of *Acacia burkittii*. The forecast from this data is that as time proceeds and older shrubs senesce

and die, there will be little or no replacement and *Acacia* will become scarce. The implications of this for the general ecology, hydrology and pedology of these areas are of course unknown. □

Purine and pyrimidine metabolism

from A. C. Allison

A symposium on Purine and Pyrimidine Metabolism was held at the Ciba Foundation on June 9–11. The proceedings will be published early in 1977 by Elsevier/Excerpta Medica/North Holland.

SO FAR most effort has gone into mapping metabolic pathways and their genetic control, and detailed information about purine synthesis in bacteria and fungi is now available. Much less is known about control in mammalian cells, but inherited deficiencies of enzymes of purine metabolism continue to yield interesting information. It has been known for some years that deficiency of hypoxanthine-guanine phosphoribosyl transferase leads to excessive uric acid excretion and abnormal function of the central nervous system, including compulsive self-mutilation. This enzyme catalyses one of the salvage pathways of purine synthesis: the other depends on adenine phosphoribosyltransferase, and the clinical manifestations of deficiency of this enzyme in three patients, belonging to two families, were described by A. Simmonds (Guy's Hospital, London), working in collaboration with K. J. van Acker (Antwerp) and P. Cartier (Paris). In these patients adenine cannot be reutilised and is metabolised to 2,8-dihydroxyadenine which, being very insoluble, crystallises in the urine. The resulting stones can be confused with uric acid stones. The children have no neurological defect and respond well to allopurinol treatment.

Other inherited defects of purine metabolism are associated with immunodeficiency. Purine nucleoside phosphorylase deficiency affects mainly thymus-dependent lymphocytes, whereas adenosine deaminase deficiency affects bone marrow-derived lymphocytes as well. W. N. Kelley (University of Michigan, Ann Arbor) reported that adenosine deaminase is present in a highly active small form (molecular weight 36,000), an intermediate form (114,000) and a large form (298,000). In human lymphocytes

stimulated by mitogens T. Hovi (University of Helsinki) has found an early and sustained increase in *de novo* purine synthesis, and immunosuppressive drugs such as azathioprine and methotrexate inhibit this pathway.

These and other observations raise the possibility that purines can control not only purine synthesis (already well established) but also pyrimidine synthesis and even nucleic acid and protein synthesis. For example, when purine nucleotide levels in mammalian cells fall, nucleolar polymerase A activity declines and precursor ribosomal RNA is no longer synthesised. ADP-ribosylation of proteins may represent a major controlling system.

Another new idea that was discussed was the role of purines, especially ATP and adenosine, as neurotransmitters. This was enthusiastically expounded by G. Burnstock (University College, London) and could have important implications in neurological diseases related to purine metabolism. □

Magnetic dating

from a Correspondent

FOR more than two decades, palaeomagnetists have been studying the magnetisation of rocks in order to construct polar wandering curves and to define the nature of recent secular variations of the geomagnetic field. Although this work is far from complete, the interest within the subject is turning increasingly towards using such observations in reverse, that is to use the magnetic properties of sediments, archaeological artefacts and so forth, as means of dating the different processes which have affected these materials by comparison with the increasingly well established magnetic chronology. This changing emphasis has been indicated by two recent meetings in the Department of Geophysics and Planetary Physics at the University of Newcastle upon Tyne. One was concerned with the possible application of palaeomagnetic techniques to sedimentological problems (held on May 14) and the other (held on January 8-9) with the status and development of the application of the same techniques within archaeology.

The meeting of geological interest was concerned chiefly with the formation of haematite within sandstones. It is well known that it takes some 5 to 10 Myr for rock debris in modern desert environments to change in colour to the reds associated with ancient desert sediments. B. Waugh (University of Hull) reported evidence

on the different reactions involved in this breakdown, which has obvious implications for the time at which the haematite is formed and therefore the actual date of the magnetisation compared with the rock itself. Many red sediments seem to carry a magnetisation which is unrelated to this coloration as the pigment is too fine grained, but D. W. Collinson (University of Newcastle upon Tyne) presented convincing evidence that in some red beds, the stable magnetisation is associated with the red pigment rather than detrital particles. Studies of Devonian sediments from Scotland, described by P. Turner (University of Aston) and R. Archer (University of Newcastle upon Tyne) showed that haematite formation in such desert sediments can be delayed for as much as 100 Myr, the diagenetic changes presumably being inhibited by a high initial organic content. If this is so, then magnetic dating of the formation of the haematite may also provide the age at which the release of liquid hydrocarbons from these lacustrine sediments finally ceased.

M. Noel (University of Newcastle upon Tyne) discussed the way in which the magnetic remanence and fabric change during deposition, thus leading to an improved understanding of the sedimentological processes and determining the original fabric of a rock before it is compacted and subjected to tectonic stresses. There was also some discussion of the way in which palaeomagnetic techniques could assist in the dating of dolomite formation (M. Tamar-Agha, University of Newcastle upon Tyne) and the identification of past environments, such as the determination of current flow when more standard markers are absent.

The archaeological meeting was initially concerned with instrumental developments, reported by participants from the University of Newcastle upon Tyne, that now make possible much faster measurement of the remanence (L. Molyneux) and other magnetic properties of materials (A. de Sa), but also mean that a wide range of materials (D. H. Tarling) previously regarded as virtually non-magnetic, can be examined for their potential use in archaeomagnetic studies. Results from Bulgaria (M. Kovacheva) and Japan (K. Hirooka) were particularly interesting as they seem to indicate a synchronicity of behaviour in both areas. If this is confirmed, it suggests that a significant part of the secular variation of the geomagnetic field is attributable to 500 and 1,500-year wobbles of the main dipole. R. Thompson and N. Abrahamsen (Universities of Edinburgh and Aarhus, respectively) described studies of sediments, particularly in present-day lakes, which sug-

gest that other components of secular variations are very localised and not attributable to the westward drift of the non-dipole field.

The meeting, however, was not so concerned with the implications of these studies for the origin of the Earth's magnetic field, but mainly with the ways in which sediments, although not ideal recorders, can be used to monitor geomagnetic changes and thus can be used for archaeomagnetic dating. One surprising feature was that sand-sized sediments seem to be better recorders than fine clays over archaeological time, as A. J. Clark (Ancient Monuments Laboratory) reported, although the opposite seems to be true of geological deposits. There was discussion of the implications of geomagnetic changes on the climate (N.-A. Möner, University of Stockholm) and production of ^{14}C (J. Klein and H. Tauber, Universities of Pennsylvania and Copenhagen, respectively). Some correlations seem to exist, but it is difficult to determine how these processes interact with each other. Changes in the total magnetic moment of the Earth should only have minor effects on the ^{14}C inventory and it seems possible that any climatic response to changes in the height of the ionosphere may be the main agent in varying the interchange of carbon between its different reservoirs.

Both meetings tended to concentrate on the significance of the increased use of sediments in palaeomagnetic studies. There are obviously major problems in understanding the complex processes involved during deposition and it will be increasingly possible to study such behaviours magnetically, but it is also obvious that extreme care has to be taken in the use of sedimentary magnetisation for precise determination of previous geomagnetic properties. □



A hundred years ago

WE publish below a correspondence which we cannot but regard with the greatest satisfaction; Government has at last seen it to be its duty to act upon the recommendation of the Duke of Devonshire's Commission, and make a substantial contribution towards the endowment of pure scientific research. We need scarcely remind our readers that from the first we have maintained that such endowment is the duty and interest of civilised states.

from *Nature*, 14, 185, June 29, 1876

200 years of American science

Reflections on 200 years of science in the United States

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In spite of its obvious importance today, the history of the sciences in the USA is usually neither the subject for research nor part of courses taught by non-American historians. Nor is this history known and understood, particularly in Europe. This is in marked contrast to an avid interest in current US science policies and developments, which cannot be wholly explicable without an understanding of their historic origins. Broadly speaking, these origins are US variants of certain western European traditions resulting, as it were, in cults of knowledge, nation, and people. Science and scientists have flourished, but uneasily, in the resulting environment.

EARLIER this year a British historian of science wrote to me to say he was giving a few lectures on the institutional history of American science before 1914 in his course. I was astonished. Hardly any non-American historians do research or display interest in science in the USA; presumably their courses reflect this. An East German has a few pieces dealing with astronomy; a Dutch historian this year published an article on a nineteenth century American physicist; a few years ago, British geologists did a multi-volume history of geomorphology, a field they defined as overwhelmingly derived from US geologists.

In contrast, there is no shortage of writings and courses outside the USA on science policy or sociology of science with strong emphasis on recent events in America. Nobel Prizes are counted, statistics marshalled, and very current trends analysed. In this dispensation, science in the USA starts with a contemporary occurrence—the Mansfield Amendment, Sputnik overhead, the atom bomb, or the arrival of refugees from Hitlerism. But research and development in the USA is important on a global scale. It did not spring up overnight but was the result of a complex, fascinating historical metamorphosis. In 1776 the British North Americans, south of former French Canada, had one great investigator, Benjamin Franklin, and a handful of others we can denote as scientists. By 1900 the new nation had essentially evolved a vigorous scientific community with many of the basic structural and functional characteristics so evident today. This included strong commitments to fundamental research in various fields. In the present century these characteristics produced what is now so visible. It is a meaningful historical movement, not simply a banal success story, and deserves critical scrutiny by scholars. Rather than give a recital of great names and notable contributions, or genealogies of institutions, let us consider the sciences in America as a set of historiographic problems particularly worth studying in comparative prospect.

Influence of De Tocqueville

Americans and Europeans are still influenced by De Tocqueville's *Democracy in America* (1835). The work is best described as a hortatory essay to the French in the form of an account of a mythical country perversely given the name of a real republic on the continent of North America. Noting the absence of the usual landmarks of European history—kings, churches, nobles, castles, dynastic squabbles and sectarian wars—De Tocqueville concluded that the mechanism of American history was different, a vast arena for the smooth workings of impersonal dynamic laws. He approved this lack of romance, drama, and great trends because of the desire to avoid the disruption of revolutions. This view recurs in the influential writings of Henry Adams, the descendant of Presidents who cast a jaundiced eye both on his society and on the consequences of science. That he thought America and China were alike in this sense says much about his insight into both histories. Like the immigrant myth of gold in American streets to explain prosperity, this Newtonian line of reasoning aborted analysis; change simply occurred, obviating any need for study of the prior actions of men and institutions.

De Tocqueville noted the presence of energetic go-getters intent on acquiring wealth, and he met no savants, devotees of abstract thought who required a niche immune from daily pressures in a hierarchical aristocratic society. A democratic society was technologically oriented and indifferent to basic research. In fact there were scientists in *ante-bellum* America. Someone like Nathaniel Bowditch, the translator of Laplace, or Joseph Henry at Princeton, might have dented his certitude. Or going forward in time to a school chum of Henry Adams, the mathematical physicist turned idealist philosopher, Charles Sanders Peirce, was every bit a grand savant. And there were others.

De Tocqueville did not say there would be no science nor great pure scientists in the United States. Applied science would yield bits of basic science—that is, he was of an older view that did not see basic science as logically and chronologically anterior to technology. And by chance a great pure scientist might arise in the American republic. De Tocqueville believed that pure science ideally required the individualism, the drama of an aristocratic order. Although he was wrong in predicting a steady-state country (and Henry Adams in seeing the heat death of entropy), De Tocqueville had grasped an important truth. The western European ideal of a value-free science independent of society would take different forms in the USA. One can argue the extent to which this ideal was realised in Europe; the structured society of its nations, at the very least, permitted the illusion of realisation. Great men, ideas, great events existed, at least figuratively, in isolation. In the USA, their analogues were often bathed, if not immersed, in social,

political, economic, and ideological realities.

To this day, many American scientists incline to the view that their society is indifferent, if not hostile, to basic science, in spite of ample evidence of the growth over time of support for research, even of the most abstruse nature. A proper perspective on this peculiar attitude is a comparison with literature, music and art. Once they too were presumed blighted by the American environment. A democratic society could support popular or vernacular cultures but not high cultures. No one makes this accusation any more in art, literature, or music. They flourish in symbiosis with the popular culture. Only in science is there uneasiness and sensitivity, obviously a phenomenon of great importance.

'Old West' influence

Early in the 1850s the aged Alexander von Humboldt wrote approvingly of American efforts in a wide range of physical and biological topics related to geography. Because he wanted Americans to receive greater international visibility, Joseph Henry of the Smithsonian Institution suggested preparing what eventually became the Royal Society's catalogue of scientific literature of the last century. As Henry originally limited the work to the exact sciences, he clearly had more than scientific geography in mind. Although the first series of the catalogue to 1863 disclosed that roughly 5% of the authors were from the USA, Europeans were not impressed. By the end of the century American activity in the earth sciences loomed large. Although an extension of Humboldt's view, geology reeked of the romance of the Old West, so attractive to generations of man-boys on both sides of the Atlantic. It did not hurt that the leader of the geological community in America was John Wesley Powell, whose hand was lost

at Shiloh in the Civil War and who had first explored the untamed Colorado. Powell's other role as a pioneer ethnologist only heightened the effect. Although very creditable, the scientific exploration of the West was like the rumoured gold in the streets, something waiting there. It did not impress those interested in mathematical and experimental fields. But only by 1951–52, did a French geologist, Emanuel de Margerie, produce his two volume *Etudes américaines*. A few works noted that astronomy had become a very active discipline in the USA by 1900.

Shortly after that date, a significant act of recognition occurred. Reacting to the founding of the Carnegie Institution of Washington and the Rockefeller Institute, the Germans founded the Kaiser Wilhelm Gesellschaft. In the years before the First World War, a number of German observers commented favourably on the quality and scale of the sciences in the USA, warning against the threat to German hegemony in research. Apparently, the familiar competition of Britain and France was less worrisome. One of the Carnegie beneficiaries, the astrophysicist George Ellery Hale, added Rockefeller funds and built the great 200-inch telescope at Mount Palomar. That great instrument and E. O. Lawrence's pre-Second World War cyclotron symbolised science in the USA to many on both sides of the Atlantic. Large scale reflected the natural endowment; great instrumentation reflected the supposed American propensity for 'practical' devices, not for the abstruse theorising of true savants. Attributes of Hale and Lawrence reinforced these stereotypes. Hale was a fabulous promoter and organiser, a veritable Morgan or Rockefeller of science; Lawrence, also a manipulator, went on to invent a television system. Usually overlooked were scientists of a different mould. A contemporary of Hale's, the great geneticist Thomas Hunt Morgan used the fruit fly in a series of austere, rigorous experiments. With a passion for scientific exactitude, he shunned empire building and applications. When Hale and Robert A. Millikan invited Morgan to the California Institute of Technology, they initially envisaged a programme of science applied to medicine; Morgan insisted on pure research. Although the USA was a world power in the sciences when Morgan went to Pasadena, a generation passed before a sustained serious historical research began and then largely because of the events of the Second World War and their consequences.

The emergence of American science

This 200 years of history is not explicable by any assumptions of American singularity. Like the nation, the sciences in the USA are part of western civilisation. The thirteen rebellious colonies were a provincial outpost of this civilisation. It is not wholly accidental that the first college in this country was founded in 1636 in Massachusetts Bay Colony in a village named Cambridge. Modern science originated in western Europe and developed an eastern wing in Russia and a western one in the USA. In spite of the obvious influence of British (and French) science, the most interesting comparisons and relations of the research scene in these former colonies of the UK are with Germany and the Soviet Union, a point to which I will return.

Because of its origins as a provincial outpost of western Europe, the USA was never properly described as a young country, that is, a country lacking the kind of history associated with the cultures of the European nations. More precisely, the USA had never experienced a youth consisting of dynastic and sectarian violence, a rigid class structure, and an all-powerful established church. It was born to adulthood in the sense of having many of the trappings of modernity. To conservatives, the lack of the usual traditions and their detritus was a basic flaw; to liberals and radicals their absence was a glorious opportunity. With some exceptions, both were sceptical about developing a high culture of science in a democratic environment.

In 1776, the USA was not a blank slate. Selectively, patterns of life and thought of seventeenth- and eighteenth-century

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'Major Powell inquiring for the water pocket.'
Kaibab Plateau, near the Grand Canyon.
Photograph by John K. Hillers, Powell Expedition,
1871–75.

Europe flourished and persisted; in time they became Americanised, diverging from a western Europe undergoing its own reactions to intellectual and economic changes. At the same time, a kind of feedback from these European developments prevented any gross cultural split between the populations on both sides of the Atlantic. In the USA there was an avid pursuit of the European printed word. Even modest cities of the Mississippi valley in the *ante-bellum* era possessed a good number of books and journals. Young scientists visited Europe, and many went on to study at overseas universities. A Louis Agassiz came in 1846, as did many others in the last century. The German emigrés of the 1930s were simply an instance in a two-century-old process. In 1973 about 6,000 scientists and engineers emigrated to the USA. At all times in its history a minority of the scientists were foreign born.

Scientific heritage

The colonists in revolt insisted they were only asking for their just rights as Britons, even though most in the UK probably did not see the rights in that way. Facing western Europe, American investigators regarded science as part of their heritage; ruthlessly and cynically they brought over the ocean whatever they wanted of this scientific heritage, disregarding any complaints about the European nature of the best of science. Being provincials in the eyes of western Europe, they were often infuriated by patronising treatment. In fact, American scientists were still vexed by patronising Europeans as late as the years between the two world wars. Yet, from the very beginning, American investigators wanted nothing as much as the good opinions of their peers in Europe; at the same time many strongly desired to present their results as a symbolic return to Europe for the great gift of science.

Because of this ambiguous relationship with European science, the American scientific community developed a differing pattern of nationalism and internationalism than their overseas counterparts. Let us disregard the fatuous cliché that "The sciences were never at war". It is a meaningless truism applicable to sets of concepts and data, not communities of humans. Real, live scientists usually rise and salute when the flag is raised, especially during wartime. Even in peace, national differences and rivalries influence the behaviour of scientists. Being outside the rivalry between the French and Germans after Sedan and not sharing the British anguish that Perkin discovered the dye but Germany garnered the industry, American scientists could work very comfortably in an international setting. At the same time, national interests were never totally absent.

Another differing pattern arising from colonial roots is the development of a cult of the people, substituting for king, nobility and gentry. In a post-First World War movie about a hack senator trying for the presidency, William Powell in the title role makes a campaign promise to give a Harvard degree to every American at birth. Mass culture, like dreams, sometimes disclose suppressed beliefs. Just as scientists (and conservative critics) were uneasy about the lack of a proper hierarchy and widely agreed on norms, the American public had qualms about status based on education. Contrary to popular belief, the nation inherited a class system from Europe and still displays a social stratification based on wealth. Contrary to De Tocqueville, quite a number of families retained high status over many generations, in some instances even constituting a patriciate. This is in spite of a significant degree of upward mobility in the past for all groups with the notable exception of those of African descent. Yet classlessness is widely assumed in the form of crowding to a middle position.

Education

Education can provide a status countering classlessness. Traditionally, expertise is highly valued here. At the same time, it is one of the glories of American history that the learned have much power and honour but lack the certainty of an

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assured niche. When historians turned to the history of the sciences in America, they noted that scientists wanted an elite, meritocratic basis for their group, often rejecting the validity of public intrusion into their affairs. Filled with such historiographic problems, I spent a year in Britain in 1964–65 studying British analogues of my favourite nineteenth-century American scientists. An acquaintance in the Civil Service, now deceased, invited me to lunch at the Athenaeum Club. As I looked around, he said, "This is like your Cosmos Club in Washington". But I knew differently. Just a few days before I had read in manuscript a letter from an officer of the Royal Society to a council member, then little known. Charles Darwin was invited to the Athenaeum for lunch to discuss a matter before the Society. From my readings of many manuscripts, I had already concluded that in comparison to these British scientists, my American 'elitists' were raving egalitarians. Even if they had not been infected with republican or democratic principles, a continuing establishment control was not feasible given the size and complexity of the USA.

Just as wealth conferred status over generations, so did education and intellectual attainments. Family groupings of scientists appeared in the last century. For example, modern geodesy was brought to the USA by the Swiss-American Ferdinand Rudolph Hassler. A generation later the Nova Scotia born mathematical astronomer Simon Newcomb married a Hassler. Their daughter became the wife of W. J. McGee, the geologist and anthropologist who was one of John Wesley Powell's lieutenants. Charles Sanders Peirce's father and brother were professors of mathematics at Harvard. J. Willard Gibbs' father (of the same name) was a professor of languages at Yale. William James' father was a Swedenborgian philosopher, undoubtedly a surprise to his teacher, the physicist Joseph Henry. A present foreign Fellow of the Royal Society is a third generation scientist.

These family groupings have never constituted an intellectual aristocracy because of the rapid growth of the scientific community over the past two centuries. A founder of the Institute for Advanced Study, the mathematician Oswald Veblen's

father studied physics at John Hopkins during its golden age; he later taught at a midwestern university. Oswald's uncle Thorstein became a great social theorist. They were a family of Norwegian immigrant intellectuals. A small town in South Dakota produced two notable physicists, E. O. Lawrence and Merle Tuve. The so-called new immigration from eastern and southern Europe by now has yielded scientists of Jewish, Italian, Slavic and other ancestries. In spite of the persistence and importance of a few families as sources for recruits, the scientific community has never been a closed group. The newest immigrants from Asia are entering the various disciplines. By now about 5% of science and engineering doctorates are of Asiatic origin. Fifteen years of consciousness raising may very well result in an inflow of Blacks, women, Chicanos—even some more American Indians.

In the USA, scientists are overwhelmingly a middle class group. In spite of individuals from the upper class and some from the lower, becoming a scientist until recently was a means of going from the lower to the upper middle class. At present such social mobility seems on the wane. If true, it is all the more important to study that portion of the middle class producing the bulk of the professional classes which provides an essential element of stability in the USA. Relatively little is known about such families in contrast to concern for the genteel culture of the patriciate or the pains and traumas of the poor and the outcast. As scientists are less numerous, they are more accessible to research than other professional groups. Where family influences are lacking, we still do not know why a small minority of middle class youngsters in the past opted for scientific careers, until very recently a choice of limited remuneration and power.

Perhaps the youngsters acted because they, like most Americans, adhered to a cult of knowledge. Here the comparisons between the USA, Germany, and the Soviet Union are important. For more than half of its history, the USA was unknowingly in a race with Germany. Early in the eighteenth century, nearly 150 years before the UK, the Prussian state made elementary schooling compulsory and universal for males. With the later expansion of German universities, the British were fated to be overtaken. The Germans trained large numbers for particular niches in their society, each niche with a specific place in an elaborate hierarchy. The professoriate were an elite of elites, having gone beyond the doctorate in their habilitation, a point the Americans did not quite grasp when graduate schools arose under the influence of German higher education. What American scientists did note was the high status of the German professoriate. In Prussia each chair holder was a higher civil servant. American scientists often hungered for the same kind of assured high place in their own nation.

Throughout the nineteenth century Americans expanded all levels of their education system; in retrospect, they were trying to match Germany. The statistics are not very clear but, apparently, around 1900, the time Germans became conscious of an American threat, the USA had surpassed Germany in numbers, at least. Quality was another question. American universities were largely viewed as an extension of the democratic thrust to provide a responsible electorate. Some institutions viewed themselves in a patrician sense, as training cultured gentlemen for service to the society. Typically, even the mass schools regarded themselves as engaged in both cultural uplift and in a service role. The sciences served both purposes. Mass plus quality tended to become the ideal.

A number of examples. In the middle of the large state University of Illinois at Urbana before a library with nearly four million books is a plot of corn planted to remind all of the university's origin as a land grant school for agriculture and mechanic arts. When the University of Chicago opened its doors in 1891, it was a great university. This oft repeated judgment refers to its scholarly research strength. But its President, W. R. Harper, at the same time launched an extension programme of correspondence and evening courses. The

Professor of Mathematics at Chicago, Eliakim H. Moore, the first pure mathematician elected to the National Academy of Sciences, received an honorary doctorate from Göttingen early in this century. He conceived his authority to extend from research in pure mathematics to supervising the training of elementary school teachers of arithmetic. Moore, Chicago, Illinois—like many other American institutions and individuals—wanted theory and practice, culture and service, elite and mass somehow combined or in juxtaposition.

The Soviet Union and the USA are probably the only remaining countries still believing in some form of the idea of progress, a Marxist version in one, and Enlightenment variant in the other. The two countries have curiously parallel histories in the sciences. Both were Humboldtian because of the need to manage their large land masses. Both turned engineering into mass professions of high status. The Russians have won the numbers race in that category, a more serious fact than mere heft of armed might. The USA lagged behind Russia in pure mathematics, and may still. Russian universities have a much different role in the sciences. Centralisation is greater in the Soviet Union, whereas egalitarian instincts limit centralisation in the USA. Our society has a primal urge to replicate anything deemed desirable. In addition, strong anachronistic sentiment for rusticity ultimately works against the big. Rusticity is something the Russians shun.

Besides the idea of progress, the cult of knowledge in the USA has other traces of the seventeenth and eighteenth centuries now largely absent from western Europe. By establishing much of the broad form and content of what now designated science, the Scientific Revolution defined some concerns as non-knowledge and started segregating the high culture of the sciences from the vernacular culture. But the useful arts partially escaped this exclusion until the nineteenth century. At the same time the casual amateur and the modest practitioner were increasingly displaced by the savant. (Significant scientific amateurism probably survived longer in the UK and the US than in Europe.) The scientific revolution gave form to a previously rather loose, amorphous conglomeration; and the Industrial Revolution hardened the new pattern.

Much of this process had by-passed the British North American colonies. The vernacular, the non-science, the amateur and the applied, survived and flourished after independence when the high culture of science began a slow steady growth. Europeans established a scheme of things in which there was a congruence between social, intellectual, and institutional hierarchies. Not a perfect congruence, but enough to avoid many of the problems of the Americans. On the western side of the Atlantic, the heritage of the past and the thrust of historical development did not neatly separate grand savant and practitioner; theoretician and earnest mechanic; the abstruse and the vernacular. They were scrambled together. A persisting tension developed between the scientific elite and their perception of fundamental research and the mass community and their thrust for diffusion of knowledge (often older and sometimes applied). As a result, the cult of knowledge in the US encompassed a research ideal, but not a basic research ideal in spite of all the exertions of many generations of scientists.

In this research ideal was a blurred boundary between theory and practice. Originally, religion provided a basis for pure research. The idea of design presupposing the existence of a Designer readily yielded a drive for research untrammelled by sordid motives. Secularisation purged the theological mission, leaving a pure research ideal. At the same time the old, general notion of the utility of knowledge was elevated by this association with a designing deity. All research carried out His purpose and disclosed His will. Only overly rationalistic, sensitive scientists worried about the problems of defining discrete forms of research styles and goals.

Although a happy compromise, even before the Civil War, American scientists launched a counter strategy, to fractionate off basic research by establishing enclaves largely immune to the national tendency to conflate theory and practice.

Higher education often served as the site of these enclaves. A notable recent example is the Institute for Advanced Study. As it were, the scientists were levying an overhead charge for theoretical science against the gross national product. Many were uneasy about the more common mode of supporting theoretical science, which also appeared before the Civil War. This is to define applied missions so as to necessarily include a basic research component. In the 1920s Harvard's E. H. Hall (of the Hall Effect) corresponded with C. J. Davission (a future Nobel Laureate) of Bell Telephone Laboratories. Impressed by Davission's work, Hall sent the head of the Laboratory, H. D. Arnold, a letter praising the company's enlightened support of basic research. Nothing of the sort was intended, Arnold replied; we are simply interested in ways of increasing the supply of electrons. Sweeping mandates like this are widely present in so-called mission-related research. Believing sincerely in their approach, the scientists still argue in favour of broad definitions of applied missions. But many also favour protected enclaves for basic research not subject to the vagaries of economics, international affairs, public health policies and the like. Yet, it is naive to hope for and expect immunity from the ebb and flow of history.

In spite of \$32 billion for research and development in 1974, in spite of over a half million engaged in research and development, in spite of \$4 billion for basic research, US scientists are still uneasy about their place in society. There is an overwhelming cult of the nation with dominating symbols—George Washington, the flag, the national anthem, Abraham

Lincoln, the Statue of Liberty. Science is not represented among the symbols of the cult of the nation; even technology once had the iron horse and Thomas Edison. And our country has just gone through ten terrible years raising nagging doubts about progress. Largely because of the anti-science quirks of Lyndon Johnson and Richard Nixon, inflation has eroded the real strength of the national research budget.

From the vantage point of my ivory tower, I find prospects encouraging, starting with President Ford's increase in the budget to make up some of the slippage from the Johnson and Nixon years. American scientists have always functioned well while loudly complaining. Even before the Civil War, they demonstrated great talents as entrepreneurs, improvisers and artful dodgers. Nor am I nonplussed by the predicted numbers of surplus PhDs. Except for about fifteen post-Second World War years and perhaps a few years before the Great Depression, there has always been a surplus of scientists on paper. A surplus can act as a stimulus, both on the nation and on individuals.

Whatever happens, the course of the sciences in the USA will remain an arena of tensions and ambiguities, a historical movement in which prosaic reality turns out to be a symbol, often exhilarating, sometimes grotesque, occasionally a black comedy. It has been a history of trying to have one's cake and eat it. But perhaps that is true of all of the past 200 years of our national life.

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The rise of 'prostituted' physics

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"From its origins to the present day, American academic science has been permeated by the influence of American business". The reciprocal influence, of science on business and industry, has grown dramatically in the twentieth century. The rise of industrial research and its sustained vigour during this period is charted.

"We are tired of seeing our professors degrading their chairs," physicist Henry Rowland told the American Association for the Advancement of Science in 1883, "by the pursuit of applied science instead of pure science . . . lingering by the wayside while the problem of the Universe remains unsolved"¹.*

Like other leaders of science he was struggling to create in the universities a spirit of pure research, free from the dangerous temptations of commerce. The task proved hopeless: from its origins to the present day American academic science has been permeated by the influence of American business. The reciprocal influence of the colleges and universities on business was for a long time not as important—nor could it be, given the small size of the scientific community. For example, in 1900 there were slightly over 200 academic physicists in the USA². They were already more numerous there than in any other single country, but were still too few to have much effect; the great advances academic physics made in the nineteenth century were usually put to practical use by semi-educated inventors and entrepreneurs. The number of physicists was, however, doubling every ten or twelve years. Over the next four decades academic physics, and science in general, entered

into a new relationship with American industry. It was a relationship that became essential to the strength of both partners (for a general treatment and bibliography of US industrial research see ref. 3).

It was true, as Rowland complained, that during the nineteenth century some American professors were almost too familiar with the requirements of industry. Sometimes they would give free advice, as Joseph Henry and others gave Samuel F. B. Morse during his efforts to develop the telegraph. At other times they would take a fee. American colleges and universities were chronically poor, and their typical professor could neither enjoy a comfortable salary nor look forward to any pension whatsoever; nor did he have a particularly high status in his commercially-oriented society. Small wonder if he was more likely than his colleagues in Europe to pick up some income on the side. Even Rowland, when he discovered that he had diabetes and would soon be leaving his family on their own resources, took to inventing and consulting for industry. Yet the scientists remained first and last professors, working as consultants only occasionally. This was, as W. David Lewis has said, the "putting out" stage of American industrial research⁴.

Around the end of the nineteenth century the universities grew greatly, becoming powerful, independent suppliers of

*Fuller documentation and much more detail will be found in Spencer Weart, *The Physics Business in America, 1919–1940. A Statistical Reconnaissance*, available from myself in preprint; to be published in a collection of essays on the sciences in America, edited by Nathan Reingold¹⁰. For unpublished sources this study has relied chiefly upon the manuscript, microfilm, and oral history archives of the Niels Bohr Library, American Institute of Physics, New York (AIP).

research. Industrial companies worked to encourage this development, although their contributions were small compared with the funds supplied by the governments of the various states, by tuition-paying students, and by affluent philanthropists. Nevertheless American business had a key role in this process; for example, it was from modern industrial ventures that most of the philanthropists derived their wealth.

But the universities, however strong they became, could not meet all the needs of the science-based industries which were growing rapidly around the turn of the century. C. E. Kenneth Mees, called by George Eastman to create a laboratory within the Eastman Kodak company, later explained why it was difficult for a company to have all its research done in universities. Industrial research requires persistence along particular lines of inquiry, he said, with specialised apparatus and training; this was difficult to obtain in a university and conflicted with the needs of teaching. Moreover, "the universities do not understand the requirements of the manufacturer and the manufacturer distrusts, because he does not understand, the language of the professor".

The idea of solving these problems by transplanting academic scientists into business organisations seems to have originated with scientifically-trained men already working for the manufacturers. Industrial laboratories of a sort had existed for years, originally for quality control and testing, a little later for engineering and development work to improve existing products. But in 1900 General Electric established a new sort of industrial laboratory in which research was no longer tied to the day-to-day requirements of production (Fig. 1). The GE laboratories were followed within a dozen years by a number of others, notably those of Du Pont, the American Telephone and Telegraph company, and Kodak.

The professors, following Rowland's tradition, were less than enthusiastic about this development. Frank Jewett, who eventually became president of the Bell Telephone laboratories, recalled that his university mentor Albert A. Michelson "thought that when I entered industrial life . . . I was prostituting my training and my ideals"⁶. Such attitudes made it more difficult for industries to hire good staff. When GE tried to bring Willis R. Whitney from the Massachusetts Institute of Technology to head their new laboratory, they had to promise him that he could split his time between GE and MIT. After a period of commuting back and forth Whitney became so absorbed in the challenges of industrial research that he gave up his professorship⁷. This turned out to be a common experience:

Fig. 1 The first research laboratory of the General Electric company—a barn behind C. P. Steinmetz's house, about 1900.



young scientists who moved reluctantly into industrial work soon found that it caught all their interest. Mees even warned that a lab director should take steps to keep his workers involved with the progress of pure science, lest they become too narrow⁸.

If industrial research was clearly established in the USA in 1910, it still could not be called a major movement. But by the 1920s broad changes were becoming apparent. There was, as one physicist noted with some distaste, an enormous advance of applied physics—in industry, in government laboratories, even in the universities themselves—a "fever of commercialised science"⁹. In 1913 physicists who were located in industrial labs made up only about a tenth of the membership of the American Physical Society. In 1920 they made up a quarter of the membership and this advance took place while the total membership of the society was doubling (my calculations, taken from *Bulletin of the American Physical Society*). The proportion of papers published in the leading American journal, *Physical Review*, which originated in industrial laboratories showed a similar fundamental increase (2% in 1910, 14% in 1915 and 22% in 1920) (my calculations and ref. 10). These facts substantiate what many men were claiming at the time: the relationships between physics and industry in America were revolutionised around the time of the First World War.

The trend was already evident before America entered the war, but the war itself was widely believed to have had a central role in accelerating the transformation. Around 1917 many physicists left their campuses, but not for the Front; for them, as for most Americans, the great event of the war was a fierce intensification of industrial organisation and production. Industrialists and scientists were thrust into closer contact with one another, both at the production level and at higher levels in Washington. The result was that industrialists and physicists alike became more aware of what could be done with physics. As one industrial physicist put it after the war, "Bankers have become science-minded. They are familiar in general with the influence of scientific research on the trend of security prices." They had come to esteem science, for in "commercial warfare . . . research supplies the ammunition"¹¹.

Research no longer meant simply development work aimed at cutting costs and improving an existing product. In some industries it had become essential to do the sort of research, sometimes verging on basic research, which could produce an entirely new product or could keep a company supplied with information and patents for defence against new products developed elsewhere. For example, both General Electric and the American Telephone and Telegraph company were able, thanks chiefly to the trained physicists in their laboratories, to maintain a strong position when the new field of radio communication emerged around the time of the First World War. It was such large companies that had the main role in industrial research; GE and ATT alone employed some 40% of all the American Physical Society members in industry between the two World Wars (my calculations, the mean of the fractions at 5-yr intervals over the period 1920–39).

The presence of trained scientists also brought a company various indirect benefits. Publicity and prestige were perhaps chief among these. As one knowledgeable observer bluntly stated, "The use of real or imaginary research laboratories to back up merchandise . . . has an importance to company prestige that cannot be exaggerated"¹². This was only true, of course, because the 1920s were a golden age of scientific faith, not only among scientists and industrialists but also for the public at large. The *Nation* complained in 1928 that "A sentence which begins 'Science says' will generally be found to settle any argument in a social gathering, or sell any article from toothpaste to a refrigerator" (quoted in ref. 13).

For all these reasons a good market for trained physicists developed in industry. One straw in the wind was Ernest Fox Nichols, a prominent professor of physics who had worked for the Navy during the war and who in 1920 resigned from Yale to join GE. "The position," he informed the university as he departed, "offers complete freedom in the choice of research problems, and places at my unhampered disposal such human and material resources as no university I know of can at present offer"¹⁴. He might have added that industrial salaries left the universities far behind. Fears were expressed that the ranks of academic science would be decimated by the movement into industry.

Some academic scientists responded by defending the old ideals of pure, non-commercial research. The physicist in a college or university had his own rewards. "Financially his lot is not a wealthy one," as one of them admitted "nor socially is he high . . . [But] Close to nature and by it closer and closer every day to the Almighty God who made him, what matters it to the physicist if the days be dull or neighbouring man uncouth? His soul is more or less aloof from mortal strife"¹⁵. In short, it was all very well to go into industry if money was your object, but there was still something morally superior about the life of academic research.

In the end the schools were not drained but strengthened by the growth of industrial physics. For it was far less the established physicist who was snatched up than the new graduate. This meant that industry was supporting an increase in student enrolments which must have had much to do with the overall rise of academic physics departments between the two World Wars. Many physics teachers felt that their departments would be strengthened by attracting students destined for industry. Applied physics courses or programmes, sometimes labelled "engineering physics", were set up by physicists at a number of schools in the early and mid-1920s.

Industry took not only undergraduates trained in physics but also PhDs. Around 1900 only a tenth of the few PhDs graduating each year in the USA wound up in industry; in the 1920s about a fifth of the ever increasing number of new PhDs were going into industry (my calculations: the sample of new PhDs was drawn from ref. 16. For convenience I used Marckworth's original file cards, at AIP. Locations from *Bull. Am. Phys. Soc.*, and Cattell, J. M., *American Men of Science* (Science Press, New York, 1906-1944)). For research—where the quality of the personnel is everything—the leaders of industrial laboratories had concluded that the best workers were those with graduate training. To select and attract these recruits they had to have close contacts with the professors who were training students. This made for strong ties between industry and the campuses¹⁷.

This also gave industry a new motive for supporting research in the universities. Through such support a company could keep its eye on promising young men while at the same time keeping abreast of scientific developments. For example, Corning Glass sponsored fellowships and research at Cornell, Illinois, MIT and Purdue on problems of chemical physics directly related to glass making. Such arrangements were probably rather common. In the absence of outright fellowship or grant support, a company could still contribute in smaller ways and keep up close relations; among the acknowledgments in papers published in the *Physical Review* in the 1920s one finds frequent thanks given to companies for the loan or construction of pieces of apparatus or the use of their laboratories.

All this led some to feel that the universities were "beginning to be led by the industries, instead of vice versa" (Pupin¹⁸ apparently regarded this trend favourably). But in so far as the point was to train researchers, the exact nature of the research was secondary. Freedom of inquiry

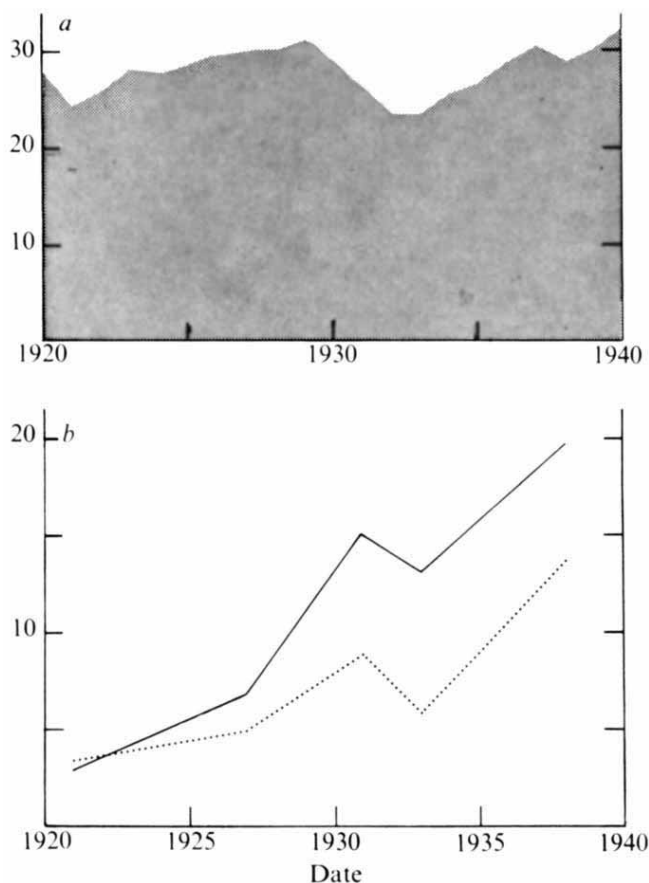
in universities was not seriously infringed on by the support industry gave; rather the possibilities for inquiry were broadened all around.

So it seemed that all was going well, and that aside from minor jealousies American physicists, industrial and academic, were advancing hand in hand in uninterrupted progress. Then came the Great Depression. When a system is struck by a heavy shock, we may hope to learn something; by listening to the seismic echoes we may study hidden internal structures. The Great Depression gives us an opportunity to do this for American physics.

The first thing to note is that academic physics did not suffer greatly. Often the professors had to accept some cut in pay, but since deflation was raising the value of the dollar, academic salaries did not drop in real terms. The research budgets of many university physics departments showed a pronounced increase in real terms through the 1930s. It is true that there were hiring freezes, and this meant that for two or three years during the worst of the Depression, few of the new PhDs could find academic jobs. But most of them simply stayed on at their schools, doing research, and found positions after the situation eased. Total US academic employment in 1938 was about 30% higher than in 1928.

The industrial physicists had a different experience, as Fig. 2 suggests. The graphs show swift growth during the

Fig. 2 *a*, Total non-farm employment in the USA, 1920-40, millions of people¹⁹. *b*, Research workers in a sample of US industrial laboratories. The figure for 1921 is raised by 23% as recommended by Perazich and Field²⁰. Solid line is for all research personnel, thousands of people; dotted line is for physicists only ($\times 15$). To the Perazich and Field sample I add the physicists in Bell, GE and Westinghouse laboratories; for Bell I use my counts from the *APS Bulletin*, and for the other two I use National Research Council, *Industrial Research Laboratories of the United States* (compiled by Hull, C.) (National Academy of Sciences, Washington, 1921-38).



1920s, a terrifying plunge during the Depression—as bad as the general collapse of employment in the USA—and then recovery. Data on budgets are harder to come by than data on personnel, but they apparently followed a similar curve. In particular, the most important single employer of physicists in the USA, ATT, cut its research and development budget by over 25% in constant dollars from 1930 to 1933, but by 1939 this had recovered and was more than 10% higher than the 1930 figure (figures for 1916–35 are from ref. 21 and for 1939 from ref. 22).

Suffering from direct and indirect pay cuts, watching friends being laid off, for a few years the applied physicists had a hard time. The director of the General Electric laboratories suffered a nervous breakdown which the Depression was said to have helped bring on²³. For the moment it seemed, as one industrial physicist declared in 1935, that “the era, or rather wave of industrial physics [had] subsided”²⁴.

But physicists in industry were spared the worst. In many laboratories research, including basic research, continued without interruption. The recovery shown in Fig. 2, so much stronger than the recovery of employment generally, also indicates a special position for industrial research. The net rise in the employment of physicists in industry from 1930 to 1938, ignoring what happened in between, was proportionally comparable to the rise in academic employment over the same interval. A large part of this swift recovery—perhaps as much as a third of it—was due to the exploitation of a new application for physics: the use of geophysics in the oil industry. Gravimeters and seismographs greatly aided geological prospecting; thus physics research helped enlarge its own field of employment.

In the 1930s as in the 1920s, the employment of new physics PhDs provided an important connection between the campus and industry. The job problems of new PhD

physicists were in large part due to hiring freezes in the academic world, but the collapse of industrial employment exacerbated their difficulties. Of 110 physicists who got their PhDs in 1928 and 1929 and whose location can be determined from the 1930 membership list of the American Physical Society, 24 were in industry and four more in government; but of 63 who got PhDs in 1934 and who are found in the 1935 list, only four were in industry and none in government. The rest were virtually all in academic institutions (my calculations from ref. 16). This decline did not escape the attention of the professors who sent the students forth.

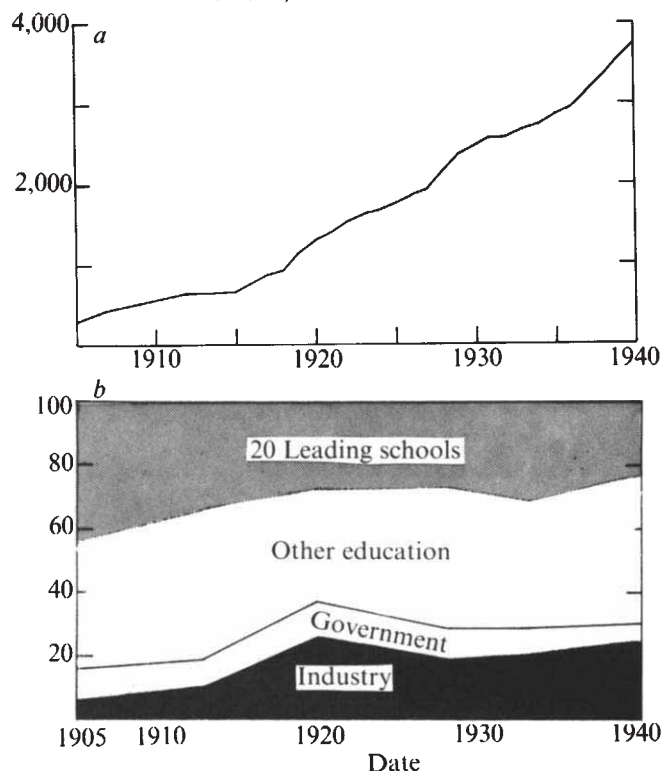
Academic physicists noted that there were applied fields to which physics could contribute but in which industry did not seem to be using the opportunity. Professors began working to persuade industrialists to employ physicists, and to train physics students so that the industrialists would want them. “A going concern must find a market for its product,” one academic pointed out, “and this is true of the profession of physics”²⁵. A leader of the physics community was equally blunt: “The number of teaching jobs available for physicists will always depend on the number of students taking physics. We know that the number of these students will increase if it becomes clear that there are industrial opportunities for them”²⁶.

Critics, industrialists and professors alike, charged that teaching was directed too much towards producing a new generation of teachers. The introduction of modern theoretical work into American university physics, which had been a prominent trend in the 1920s, did not impress these critics; what the physics graduate needed, they said, was less work with quantum mechanics and more with classical physics and a slide rule. Physicists feared that this sort of training was being usurped by the engineering faculties, whose graduates more and more resembled the product of the physics departments. To see how the universities were meeting these problems, physicist Henry Barton wrote letters to a number of physics departments at the end of 1935. He found that about half of them had taken, or were seriously planning to take, steps to increase the attractiveness of their students to industry²⁷. Not all schools joined this movement, and at any rate the majority of students continued to end up with academic jobs. But at certain schools a majority of the physics PhDs who graduated in the 1930s found their homes in industry.

We have noted that the movement to teach applied physics in the universities included some elements of a reaction against pure and theoretical physics. The conflict also came to the fore in another theatre, the physics societies. Figure 3 analyses the chief of these, the American Physical Society. Figure 3a shows the growth of society membership from 1905 to 1940. The effects of the Depression, or even the First World War, on this curve would be difficult to prove. The breakdown of the membership (Fig. 3b) shows what percentages were in various locations. The shaded areas at the top, education (including private non-profit research institutes) dominate throughout. But there is a significant change in the proportions employed by industry (the black area) and by government (the white). Their combined total grows strongly into the 1920s and then drops off. Note that because of the rapid growth of the American Physical Society as a whole, the absolute number of applied physicists in it was higher in 1940 than in 1920; but they were never again so large a proportion of the total as they were in the 1920s, the golden age of applied physics research.

People noticed that not all those who might be called physicists were members of the American Physical Society. Many belonged to the engineering societies and called themselves engineers, even though their work was in direct application of physics research. In fact, of the 1,500 or 2,000 so-called physicists in industry in 1938 only about

Fig. 3 a, Membership of the American Physical Society, 1905–40. b, Location of employment of American Physical Society members, as percentage of those whose employment can be inferred from their addresses and was in the USA. From top to bottom the areas refer to the twenty universities with strongest physics departments; all other “education” including non-profit institutions; government; and “industry” including business, medicine, and miscellaneous.



half were in the American Physical Society. The reason was that applied physicists were coming to feel themselves set apart from those in pure physics. "We did not feel at home in the Physical Society meetings," one of them recalled. "I once read a paper before the Physical Society, and I am sure the Physical Society was not interested in the paper"²⁸. These feelings had gained strength through the 1920s as the numbers of applied physicists grew and as they noticed that the society was becoming more concerned with quantum mechanics and atomic spectra than with physics in the broadest sense. Already in 1916 the Optical Society of America was founded by a group at Eastman Kodak; in 1929 the Acoustical Society of America was founded at Bell Laboratories and the Society of Rheology was founded by people interested in the study of plastic materials. In 1930 there were rumours that a Society of Applied Physics would be created²⁹. The trend seemed to be towards a complete schism in the physics community.

This was avoided by the founding in 1931 of the American Institute of Physics, which undertook operations such as publishing and public relations which were most efficiently done jointly. Throughout the mid-1930s this organisation had a leading role in bringing industrial and academic leaders together, in trying to explain the value of physicists to industry and the value of industry to physicists, and in working to persuade applied physicists to stay within the fold. Several conferences were held with joint academic-industrial participation³⁰⁻³². "To those of us who have been engaged in industrial research," one physicist wrote in 1937, "... there appears to have been a social upheaval in the realm of the physical sciences in which the researcher in practical or applied fields has been lifted from the gutter of so-called 'prostituted' science and at least his better qualities held up as ideals for aspiring young physicists to emulate"³³.

The actions of academic physicists during the Depression period, through new curricula and new organisations, shows great sensitivity in the area of applied physics. If the professors were not all wrong, then this sensitivity is evidence that any weakening of applied physics or of its ties to the academic world would have been a heavy blow to all physics.

Fortunately, as we have seen, industrial physics recovered. Towards the end of the 1930s there were even hints that the most abstract physics of the universities might have some value for industry after all. General Electric advertised for a quantum theorist to look into the fundamental quantum processes involved in fluorescence, as in fluorescent lights; people at the Bell Telephone laboratories studied the quantum theory of solids, setting out on the path that would lead to the transistor; Westinghouse went into nuclear physics, a subject which later became one of its major fields of industrial interest.

In conclusion, the experience of the Depression indicates that the greatest strength of American physics still lay in the academic sector. But the anxious reaction of academics

to the situation in industry suggests that a strong industrial research establishment had become as necessary, in the long run, to the universities as the universities had become to industry.

Finally, the situation has not changed greatly from the 1930s to the present day. For example, in 1973 some 53% of American PhD physicists worked in the universities, 24% in industry, 11% in government and 12% in federally funded research and development centres^{34,35}. Except for the growth of federally funded centres at the expense of the universities, this is about the same as the situation before the Second World War. The relationship between academic and industrial physics research in America, established during the first four decades of the century, has proved as stable as it has been fruitful.

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Population studies and the Old Order Amish

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Populations of Old Order Amish, living in Pennsylvania, Ohio and Indiana, and isolated by their strict religious views, have provided human geneticists with a rich source of data.

LIFESTYLES in the United States, as in many other countries, have changed remarkably during the past 200 yr. Technological advances, with improved transportation and nearly instantaneous communication, have probably had the most significant impact on our social structure and cultural evolution. With progressive industrialisation and the need for highly specialised

skills, these factors have contributed to the decline of relatively self-sufficient, isolated agrarian communities in which the nuclear family comprised the basic living unit. These have, of course, been replaced by large conglomerations of interdependent, amalgamated, and highly mobile masses to which the "melting pot" metaphor is perhaps more aptly applied than during the early days of settlement.

Nevertheless, certain segments of the US population have remained relatively unaffected. In some circumstances, the trend has been at least partially countered by cultural ties or economic restrictions as in the case of the ethnic and ghetto neighbourhoods of major metropolitan areas. In others, geographic barriers such as the Appalachian mountains of the eastern USA and the marshy bayous of the deep south, have effectively excluded these socioeconomic influences. Among the triracial isolates scattered throughout the eastern coastal states, racial barriers have prevented assimilation by restricting matings to individuals with a mixture of Indian, Caucasian and Negro ancestry.

The value of insular populations has long been recognised by human geneticists. Lacking the luxury of a highly proliferative species such as *Drosophila*, or the benefits of controlled breeding experiments available to a mouse geneticist, they are instead dependent on nature to provide the appropriate human population model. Human isolates, separated as they are from the prevailing cultural mores, often have at least some of the desirable characteristics of the biologist's experimental model. Many have large families, thus making available larger numbers of progeny for study. Social or religious taboos frequently limit dilution of the gene pool by restricting matings to members of the group. Such populations are often geographically concentrated due to their limited mobility and identification is facilitated by peculiarities of custom or dress.

Genetic aspects of isolates in industrialised countries have, however, received relatively little attention. Clinical investigations in Europe¹ and Scandinavia², as well as North America³ have contributed significantly to the understanding of selected heritable diseases, and, in some instances, revealed "new" disorders. Other isolates in North America have been the object of demographic⁴ and immunological⁵ studies. In 1963,

Fig. 1 North-east view from Millersburg, Ohio courthouse showing Amish horse-and-buggies tied to rails at parking meters. This blend of tradition and modern technology illustrates the accommodative attitude of non-Amish toward their unusual, isolationist Amish neighbours who, in turn, patronise "worldly" businesses without becoming assimilated.



Hostetler⁶ published the first major sociological study of the Old Order Amish whose unique features were immediately recognised by McKusick⁷ as useful for genetic studies. Through their collaboration and that of others during the next decade, the Amish have become the most extensively studied isolate group in the USA.

The Amish constitute one of several religious factions classified as Anabaptists because of their opposition to infant baptism. Unlike all other Anabaptist sects, such as the Mennonites, Dunkards, Amish-Mennonites, and Beachy Amish, the Old Order Amish hold religious services in their homes rather than in churches or meeting houses. Although this unique feature absolutely distinguishes them, other characteristics make tentative identification possible. Perhaps none is more conspicuous than their eschewal of technological innovations. Electricity and telephones are not allowed in their homes although in communities where Ma Bell has enterprisingly placed payphones in strategic locations, at least some Amish freely take advantage of the opportunity. The use of petrol engines is likewise forbidden, but curiously, steam is an acceptable, albeit rarely used, form of energy. This limitation, plus the interdiction of pneumatic tyres, has restricted transportation to the horse-and-buggy so familiar in their communities (Fig. 1).

All Amish are rural living and nearly all are farmers. They are highly clannish and have a strong interest in their ancestry and genealogical relationships. This has stimulated the publication of numerous genealogy volumes by which the ancestry of most members can be traced to the original immigrants. Perhaps most importantly, this simple folk society has somehow managed to resist assimilation and annihilation through strong internal disciplinary measures, including the practice of "shunning" or *Meidung*, by which transgressors are totally ostracised.

To the casual observer, however, personal appearance is the most striking peculiarity of these "plain-clothes people". Any form of personal adornment, including brightly coloured clothes, is strictly forbidden. All external clothing is handmade by the women from plain brown, blue or black fabrics. "Barndoor" trousers held up by suspenders and jackets with hook-and-eye fasteners are worn by the men; women's dresses are fastened with straight pins. Hair style for males is a uniformly cut, medium length, 'upside-down bowl' style (embellished with a beard without moustache after marriage), whereas females are forbidden to cut their hair. Instead, they wear it rolled up into a bun, covered with a prayer veil during all waking hours.

The Amish originated in the Canton of Berne, Switzerland, when followers of Jakob Ammann split from the parent Swiss Anabaptist sect between 1693 and 1697. Other converts came from eastern France, the Palatinate and Alsace-Lorraine. Never fully accepted in Europe, and viewed by their fellow citizens as rather peculiar, many Amish were strongly tempted by the religious freedoms promised in America. Immigration began perhaps as early as 1714 and continued for nearly 125 years. The first immigrants, mainly Swiss and numbering approximately 200, settled in eastern Pennsylvania as founders of the present group known as the Lancaster Amish. After about 1770, however, new immigrants, primarily from Alsace-Lorraine and nearby areas, found Pennsylvania too crowded and moved further west, chiefly to Ohio. The Holmes and Geauga County, Ohio Amish in the mid-nineteenth century became, in turn, the primary source of founders for the third major concentration of present-day Amish which is centered in Lagrange County, Indiana. Although Amish populations are now located in approximately 20 states, and a few recent attempts have been made to establish populations in the Honduras and South America, about 80% of the nearly 50,000 practising Amish live in the three states mentioned above. Migration and religious persecution eliminated all Amish from Europe.

The Amish are strictly endogamous, do not proselytise, and "outside" marriages with non-Amish are strictly forbidden. These restrictions, together with the limited number of founders, has led to a relatively high mean coefficient of consanguinity ($F = 0.00769$ in the Holmes County group), equivalent to

second cousins once removed. In addition, however, their limited means of transportation, subtle differences in religious practices, and, in the case of the eastern Pennsylvania and Holmes County, Ohio groups, separate origins have effectively produced subisolates. For example, eight surnames account for 81% of the Lancaster Amish population whereas 77% of the Ohio Amish have a different set of eight surnames. The continued isolation of these two communities is illustrated by data from an analysis of 2,013 Holmes County couples living in 1966: only two partners came from Lancaster County. Such isolationism is evident even among Indiana and Ohio Amish with much closer ancestral and religious ties: only 116 marital partners left Indiana to join their mates in Holmes County, evidence of separation which no doubt portends even greater genetic dissimilarities in the future.

Interestingly, in spite of their usual pacifism and strong belief in non-violence, there is considerable internal strife. New religious factions are constantly being formed and dissolved. In Holmes County, for example, two groups known as the Stutzman and Stoltzfus Amish split from the parent body in the early 1960s and marriages between any of the three groups is actively discouraged. If this practice persists, new genetic isolates may be formed.

The Amish community is primarily a spiritual one without distinct geographical boundaries. Amish farms are scattered throughout the rural communities, often sharing borders with non-Amish farms. Their religious charter, however, effectively prohibits social and cultural interaction with the non-Amish community with the result that their demographic characteristics⁸ are quite distinct. As a result of their high crude birth rate (33.3 compared with 21.2 for the entire USA in 1964), the population pyramid⁸ has a relatively broad base; 51.8% are less than 20 yr old and 28.7% are less than 10 yr old. The pyramid tapers more smoothly than that for non-Amish populations since their self-sufficient agrarian lifestyle renders them relatively independent of the cyclic effects of political, economic and social stimuli. Most men (69.8%) are farmers, at least in Holmes County where the most complete data have been collected. A further 13% are self-employed in supporting trades such as carpentry, blacksmithing, harness repairing and construction.

The Amish marry at a considerably later age than their rural counterparts (22.57 yr for a female, and 23.61 yr for males). Because of the obvious economic advantages of large families in a rural setting, and their strict interpretation of the biblical admonition to "be fruitful and multiply", children are considered highly desirable. Contraception, although not prohibited, is viewed as undesirable with the result that the female reproductive span is longer and birth intervals shorter than among other US rural populations. Among 792 completed Holmes County Amish families (in which the wife was at least 45 yr old), the mean number of pregnancies was 7.19 and more than one-third (34.8%) reported 10 or more pregnancies. The net result is an intrinsic rate of natural increase (r) of 3.019 or a potential doubling of population size every 23 yr. Among modern North American isolates, only the Hutterites were found to have a higher rate ($r = 4.1265$). This increase is not achieved for the Amish, however, because there is a substantial attrition rate among young people who are lured away by the 'outside world'. Still, it is obvious that the Amish could lose four or five times the 10–15% estimated to leave each generation, and still maintain their population size at the present reproductive rate.

Curiously, Holmes County Amish men tend to outlive their wives. The sex ratio in this population for those over the age of 55 yr is 1.216. Similar trends have been noted in other high fertility groups such as the Hutterites and northern Indiana Amish, giving rise to speculation that the emotional and psychological stresses of childbearing may have an adverse effect on longevity.

It is apparent that the Old Order Amish have several attributes that make them useful to human geneticists. Members are

Table 1 Distribution of some rare recessive genes in the Old Order Amish

Isolate	Recessive disorder
Mifflin County, Pennsylvania	Pyruvate kinase-deficient haemolytic anaemia ⁹
Lagrange County, Indiana	Phenylketonuria ^{10,11}
Lancaster County, Pennsylvania	Ellis-van Creveld syndrome ¹²
Adams and Allen Counties, Indiana	Limb-girdle muscular dystrophy ¹³
Holmes County, Ohio	Mast syndrome ¹¹
	Troyer syndrome ¹³

easily identified by their distinctive dress and customs. Their communities were founded by a limited number of ancestors, with identifiable origins. They maintain a closed population with virtually no dilution of the original gene pool. Families are large, illegitimacy rates are low, and the membership constitutes a relatively immobile society concentrated in specific geographical areas. Several additional features are of particular value to the medical geneticist: relatively stable socioeconomic values, uniform environment, high standards of living and medical care, a great interest in medicine, and strong resistance to institutionalisation of the chronically ill.

Populations founded by a small number of ancestors may exhibit an unusually large number of otherwise rare hereditary traits if the founders carried a frequency of mutants that differed widely from the parent population. If the population further remains 'closed' by excluding matings with outsiders, the purity of the original gene pool is maintained, and the restricted number of available mates results in a high rate of consanguinity. Since consanguineous couples share ancestors, they are likely to inherit at least some identical genes, and chances are good that their offspring will inherit the "double dose" of mutants necessary for the expression of recessive disorders. These conditions were apparently satisfied in the case of Amish settlements since all of those studied have at least one recessive disorder occurring with relatively high frequency (Table 1).

The limited distribution of these genetic diseases is another indication of genetic isolation. Only one recessive disorder, a "new" type of dwarfism (cartilage-hair hypoplasia), seems to occur throughout most Amish communities including Holmes, Lancaster and Lagrange counties as well as among smaller settlements in Iowa, Oregon, Kansas, Illinois and Michigan¹⁶. Either the origin of this gene was more ancient, antedating the origin of the sect, and was introduced by multiple immigrants, or the descendants of the person or persons who introduced it were unusually nomadic.

The peculiar social and cultural restrictions of Amish life also make them useful for epidemiological and population studies. Certain aspects of the distribution of cervical cancer, for example, raises the suspicion that it might be a venereally transmitted disease. Women with increased risk are those with marital instability, early coitus or early marriage, multiple coital partners and other venereal infections. Under this hypothesis, groups with low risk for cancer of the cervix would practice (1) strictly endogamous matings (to avoid introduction of the responsible agent), (2) monogamous sexual behaviour (restricting the agent), and (3) strong group boundaries over many generations. These are, of course, characteristics of the Amish, maintained and enforced by their religious taboos. Divorce is forbidden and separation is extremely rare. In either case, marital infidelity is virtually unknown, although premarital conceptions probably occur with a frequency similar to other US populations. In most cases, however, the biological parents do become married with the result that the overall illegitimacy rate is low. Among 1,098 Amish offspring of 155 couples in northern Indiana, no paternal exclusions were found by ABO, Rh and MN blood group determinations⁵. Furthermore, their strict moral standards regarding marital fidelity and their condemnation of divorce limit the transmission of other venereal diseases, according to local physicians.

Table 2 ABO blood group frequencies in three Amish isolates

Blood group	Holmes County isolate*	Lancaster County isolate†	Adams County isolate‡	Random Anglo-Saxon population ¹⁹
O	0.34	0.11	0.16	0.45
A	0.54	0.74	0.65	0.41
B	0.06	0.05	0.11	0.10
AB	0.06	0.11	0.08	0.04

**n* = 1,027; ref. 18.†*n* = 215; ref. 18.‡*n* = 892; ref. 17.

Use of the Amish to test the possibility of venereal transmission of cervical cancer is especially valuable because its frequency among them can be compared with that found in non-Amish living in the same area where medical care practices are similar and the effect of economic and environmental factors is largely cancelled out.

A retrospective study in Holmes County, Ohio, based on 10,314 Papanicolaou vaginal smears obtained between 1950 and 1966, revealed a significantly lower frequency of cervical cancer in Amish women. Among 3,606 non-Amish women, three died of cervical cancer (a frequency similar to other rural populations) whereas none of the 2,068 Amish women examined died of this disease. These data are even more interesting because the Amish have several characteristics associated with high risk groups, such as a high rate of reproductivity, absence of contraception, and a low rate of circumcision (at least among older males). In view of the similarities of economic standards and medical care practices between the two groups, it seems plausible to attribute the difference in frequencies to some aspect of social or cultural behaviour. Social isolation and reduced sexual contacts between Amish and non-Amish might, therefore, account for the reduced rate of cervical cancer if it is caused by a venereally transmitted agent.

Blood group data are widely used by population geneticists in the study of evolution and genetic similarities of various groups. To further document subisolate formation among the Amish, ABO frequencies for three communities were determined. Blood was collected systematically from the Amish isolates located in Adams County, Indiana¹⁷ and Lancaster County, Pennsylvania¹⁸. For the Holmes County community, however, extensive data already available were used by taking advantage of the fact that Amish do not have telephones. Due to their interest in medical illness and the relatively high frequency of haemophilia B (Christmas disease), Amish in this area give

blood freely. Red Cross donor cards were compiled on all citizens of the area and Amish individuals identified by characteristic surname on cards without a telephone number. ABO frequencies among the three populations, as well as a random Anglo-Saxon sample, are compared in Table 2. It is apparent that the Amish not only differ from non-Amish in ABO frequencies but they evidently maintained significant isolation from each other.

In spite of their apparently effective isolation from the external world, and evident internal stability, the Amish exhibit signs of stress that may indicate their eventual acculturation, at least to some degree, with the larger rural community. Demands for higher education and restlessness among young people seem to be increasing. Pressure for relaxation of rules against the use of mechanical devices such as tractors and automobiles by older individuals is responsible for considerable internal strife and fragmentation. At the same time, rising costs and, in some areas, decreasing availability of good farm land is forcing many to resort to marginal, non-farming vocations, bringing them into closer contact with worldly temptations. Nevertheless, their reproductivity and strong internal sanctions make it unlikely that this unique society will disappear in the near future. In the meantime, these peculiar people have provided us with an unusual opportunity to study the genetic and epidemiological aspects of human disease to a degree that will be increasingly difficult to achieve among the modern population.

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Scientific networks in the age of the American Revolution

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The channels of communication between scientists in the UK and USA during the late eighteenth and early nineteenth centuries, the growth in importance of the provincial centres and the relative position of London, are reviewed in the light of the social, cultural and political moods of the time.

"THE rights and liberties of our countrymen in America are much less dear to us than they ought to be." The words were those of a rising Manchester physician, in a June 1774 letter to a well known Philadelphia printer. The physician's

concern encompassed more than remote, colonial affairs. The situation at home also gave cause for alarm. "The principles of despotism in our Governors and of passive obedience in the people seem to be advancing by an equal and alarming progression." These opinions were shared by other influential Mancunians, including a manufacturer who wrote some nine months later to Philadelphia's foremost physician, saying "I sincerely regret, from more general considerations than my own interest, the unhappy divisions that prevail between the mother country and her colonies. Soon may they again be united, and may their quarrels like those of lovers, tend to confirm and strengthen the mutual affection which ought to subsist between them."

The exchange of such sentiments was halted abruptly by the onset of the War of American Independence. It was almost ten years before the manufacturer wrote again, marvelling "how strange and unexpected have been the events . . . (causing) so long and unfortunate an interruption to our correspondence." He went on to say that "tho' separated and become subjects of different states, I trust that every spark of affection is not extinguished, or at least that individuals will renew and maintain their pristine attachments." The hope was expressed in the formal language and cast in the individualistic terms of the period. As events were to show, the hope was also eminently realisable, and a clue as to why may be found in a third letter. It was in September 1786 that the then premier physician of Manchester confided to his counterpart in the City of Brotherly Love: "I shall be happy in every opportunity of promoting a friendly and philosophical intercourse between my countrymen and the literati of America. The arts and sciences are the offspring of freedom; and I cordially rejoice that your noble exertions have secured to you these invaluable offspring."

The scientific network

The series of letters from which these quotations come provide us with a glimpse of an important scientific network of the late eighteenth century. The correspondents—Benjamin Franklin, Thomas Henry, Thomas Percival and Benjamin Rush—were all major figures in their day, as was Joseph Priestley. Franklin's work in electricity is well known, as is Priestley's discovery of oxygen. Thomas Henry was also influential in the chemistry of the period, publishing translations of the work of Antoine Lavoisier in 1776 and 1783. Percival in his turn was active in chemistry and meteorology, and was a pioneer of mortality statistics. He composed a standard work on *Medical Ethics*. Finally, Rush was not only a chemist and physician of note, but also a leading medical and psychiatric theorist. Each of the five participated actively in one particular link in the network of provincial, rationalistic dissent.

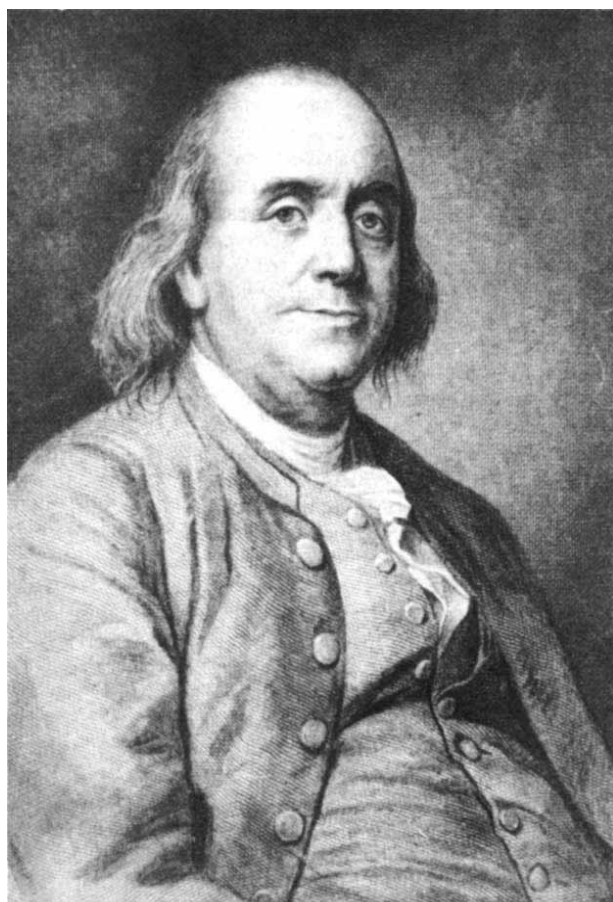
Other scientific networks, each encompassing its own appropriate linkages, were also significant in this era. Two come to mind as especially important. Most immediately and most obviously, the affairs of the Royal Society of London, and of much else besides, were quite unselfconsciously controlled by the members of a network that linked together the metropolitan elite, the English universities and certain interested and influential country gentry. One reason that Sir Joseph Banks was able to entrench himself as President of the Royal Society and thus to serve for the record span of almost forty-two years (November 1778–June 1820) was because he exemplified in himself the three elements of this central network of the late eighteenth century. The third network, connecting with but quite distinct from the previous two, was the one that centred on the scientific interests of the Scottish nation. Its members came from the ranks of Scotland's 'improving' landlords, from among the professors at her several universities and from those native-born students who emigrated to England and her dependencies in search of wider opportunity.

Analytically, we normally separate the science, the politics, the philosophy and the religion embraced by such men and involved in such networks, and treat each by itself. If, instead, we view the cultural values, social functions and intellectual commitments involved in more holistic fashion, we may understand better not only the ways in which images of science, progress and freedom were woven into the fabric of the American Revolution, but also the functions of scientific networks in the preindustrial age. The particular case of provincial, rationalistic dissent, as seen in the microcosm of the Philadelphia–Manchester link, may illuminate the macrocosm of social structure, political

change and the growth of knowledge. Equally, our understanding of the macrocosm may help us comprehend what we see revealed in the microcosm.

London as a focal point

By the mid-eighteenth century, London was Europe's largest, richest, most complex and most cosmopolitan city. In population it was soon to outrank its nearest rival, Paris, by a factor of more than two. Within the English-speaking, as opposed to European, world, London's pre-eminence was even more pronounced. No other English-speaking city was one tenth of its size. Advantages of scale were reinforced by the city's commercial, financial, intellectual, legal, political and social significance as the centre of a newly united Britain and of a dawning empire. The Royal Society of London for Improving Natural Knowledge was thus in an enviable situation. A metropolitan elite of peers, gentlemen of independent means and successful physicians, clergy and lawyers gave tone and direction to its activities. Foreign ambassadors and visiting princes attended its sessions. Lowlier but more earnest visitors from the colonies, the provinces and the lesser metropolises of Dublin and Edinburgh brought information, specimens and favourite but unwinnowed ideas. On returning home they took not only tested knowledge and new theories, but heightened aspirations and fresh models of behaviour. Benjamin Franklin was one of 53 American colonists who became an FRS. Thomas Percival, Thomas Henry and Joseph Priestley were among the larger, but uncounted, group of English provincials who successfully sought the heightened social standing and envied cosmopolitan connections implicit in the Fellowship. The subsequent ostracism of Priestley provides a vivid example of how member-



Benjamin Franklin.

ship still left one a likelier candidate for neglect than for office within what was correctly called the Royal Society of London.

Importance of provincial centres

Those on the provincial and colonial peripheries of the eighteenth century social world naturally sought approval from the centre and conformity with its values. Dissent was an alternative which was, however, sometimes as attractive as assimilation. Ambivalence between the two marked the growing mercantile communities of provincial cities from Birmingham to Boston. This ambivalence ensured good connections between provincial, rationalistic dissent and the central scientific network on the one hand, while also encouraging connection with Scottish science on the other. Those who literally dissented in central, religious values were of course, barred from graduation at either English university. Edinburgh provided a favourite locus for such dissenters. Its different devotional traditions and its own particular ambivalencies about English norms gave promise of congenial context. The varied clubs and institutions of the town and most especially its rapidly-growing university served as centres of socialisation into alternative values. Within the university itself, the medical school offered not only appropriate direction but also coveted certification for a burgeoning line of service and opportunity. Thomas Percival and Benjamin Rush both studied there. Percival later went so far as to secure an Edinburgh doctorate (of law) for his friend and mentor, Joseph Priestley (Priestley in his turn had sponsored the youthful Percival before the Royal Society).

Edinburgh-trained physicians were drawn to the new medical opportunities apparent in the 1750s and 1760s in such rising provincial towns as Philadelphia, Manchester, Liverpool, Bristol, Boston and Birmingham. Most dramatic is the case of Philadelphia. The city was beginning to develop its own intellectual life and to serve as centre for the scattered seaboard communities of British North America. It was thus the natural site for the continent's first medical school. Like Rush and its founder John Morgan, its earliest professors all enjoyed Edinburgh MDs. Custom and the reach of law as institutionalised in the monopoly power of Oxford, Cambridge and the (London) College of Physicians were sufficient to preclude such developments in England. But MDs with Scottish training played influential, if less formal, roles in provincial English towns, serving as nuclei round which social forms oriented to natural knowledge, polite behaviour and advanced political thought might crystallise. Their influence is apparent in the development of, among others, the Lunar Society of Birmingham (about 1765), the Manchester Literary and Philosophical Society (1781) and the Derby Philosophical Society (1783), as well as in the cognate cases of the American Philosophical Society (Philadelphia, 1769) and the American Academy of Arts and Sciences (Boston, 1780).

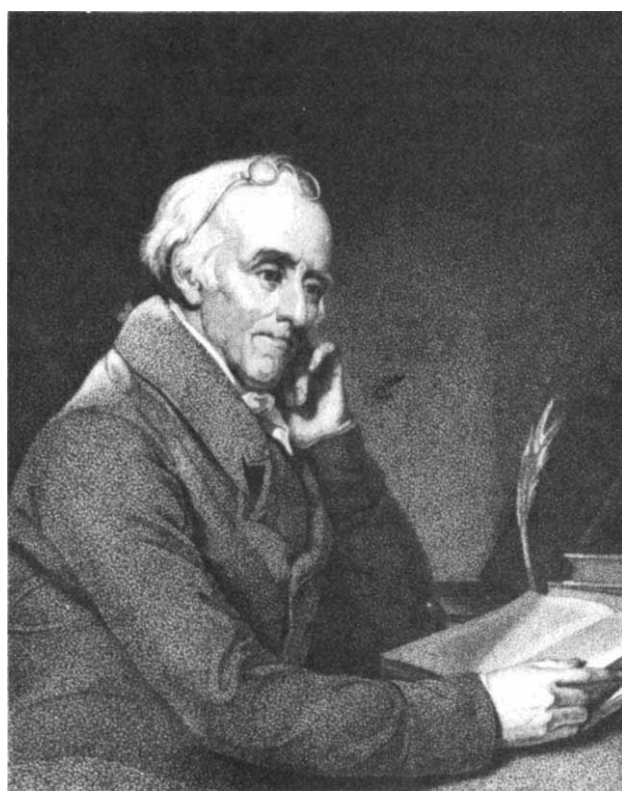
Medical men of all kinds, including apprentice-trained surgeons and apothecaries, were a key element in the growing eighteenth-century network of rationalistic, 'scientific' dissent in both its provincial and colonial forms. Family connection and religious conviction also had major roles. If Edinburgh-trained physicians were the shock troops of the movement, dissenting ministers of a Socinian turn of mind served as its theorists; the most notable of these was Joseph Priestley. Between his electrical investigations and his brilliant discoveries of ever more curious sorts of "air", he produced endless polemical broadsides on theology and served as a 'culture hero' to a generation. Priestley was directly associated not only with the Lunar Society and the Manchester Literary and Philosophical Society but also, eventually, with the American Philosophical

Society. Less spectacular but more typical was the Turner family. William Turner was a Unitarian minister and an early member of the Manchester Literary and Philosophical Society. Using this as a model, he founded the Literary and Philosophical Society of Newcastle upon Tyne (1793), which he served as secretary for over forty years. His son later fashioned a similar 'Lit and Phil' in the burgeoning industrial town of Halifax.

Other examples of the connections between medicine, rationalistic dissent, merchant activity, manufacturing concerns and family lineage spring readily to mind. The Lunar Society provided a nexus for Darwins, Wedgwoods and Witherings to enjoy polite conversation, pursue natural knowledge and forge marriage alliances. Erasmus Darwin founded another Philosophical Society when he moved away to Derby in the early 1780s, and was promptly rewarded by election as an honorary member of the Manchester Society. As late as 1817-18 his grandson, Charles Darwin, attended a Unitarian school before arrangements were made for him to follow family tradition by reading medicine in Edinburgh. Finally, we note how a group associated with the Manchester Literary and Philosophical Society even went so far as to plan a pantisocracy in Pennsylvania when, following not only the American but the French Revolution, the political climate in England turned decisively against progressive, rational dissent. The movement to Philadelphia in 1794 of Thomas Cooper, Thomas Henry, Jr and the Joseph Priestleys, both senior and junior, may be seen as a last efflorescence within these eighteenth-century forms. (The first of the foursome obtained, the second unsuccessfully sought, and the third declined, a scientific chair within the University of Pennsylvania.)

The network and the revolution

The American Revolution symbolised the coming end of the symbiosis between provincial and colonial values. The path



Benjamin Rush.

of development in the USA was to be one in which Americans painstakingly, precariously and long unsuccessfully sought to redefine "their" centre from London to somewhere—no one could agree where—in the new nation. Not until 1863 was an enduring National Academy of Sciences founded; not until the first World War did that Academy become a main voice in American science. The modes of federalism and pluralism developed in the USA contrasted strongly with the trend to centralism in British life. In the course of the nineteenth century the UK became a tightly integrated whole. The nature of that integration was neatly echoed in the new forms of science. The British Association for the Advancement of Science, founded in 1831, depended heavily for its sustenance on scientific societies spawned from the network of provincial, rationalistic dissent. Reflecting its origins, it never met in London although in its infant days it was captured, and has since been controlled, by the central elite. To say this, however, is to progress ahead of the story and we must rather return to the later eighteenth century, when an Anglo-American network of provincial, rationalistic dissent was a significant element in the world of science and culture. The letters which follow indicate the modes, styles and concerns of that network as seen in the particular linkage from Manchester to Philadelphia.

The earliest surviving communication from this link in the network is a letter from Thomas Percival to Benjamin Franklin of 1771. Characteristically, Percival encloses an offprint of a scientific publication, seeks critical commentary and concludes by saying "My friend Dr Priestley flattered me last summer with the prospect of seeing you at Manchester, and obligingly promised to introduce me to your acquaintance." It is not known how Franklin responded or when the two eventually met. The sustaining network of shared friends, values and commitments however, was such as lead to a ripening correspondence. Two years later Franklin wrote with commentary on census attempts, and remarks on climate and health. The following year Percival in his turn acknowledged receipt of "a packet of papers on the American affairs." He was quick to point out that he had "distributed the pamphlets amongst persons of the first consequence in this town and neighbourhood. They have already circulated through a considerable number of hands and cannot fail of making some useful impressions." Percival was equally quick to "inclose a little paper" of his own "extracted from a long memoir which I shall soon send to . . . the Royal Society". The easy turn from political affairs to scientific enquiry highlights how, for Franklin, Percival and other members of the network, similar values underlay the two pursuits.

By this time Thomas Henry and Benjamin Rush were also active within the Manchester-Philadelphia strand of the network. In 1774 Henry sent Rush his book on the properties of magnesia. Rush returned the favour, conveying the first volume of the *Transactions* of the American Philosophical Society and announcing that he had proposed Henry as a member of that fledgling body. The parallelism of developments in the two cities, and the way in which the network was underpinned by common concerns with trade and with medicine may be seen in Henry's response. After dilating on the "very pleasing proofs of the progress science is making on your side of the Atlantic," he continued that "happy as I am at Manchester in the society of several men of real genius, and most diligent investigators of nature, yet I must think that an inhabitant of Philadelphia has ample reason to congratulate himself on being situated in a city where the literati so much abound. One of your Society, Dr Williamson, was at Manchester in January and seems to be a man of very extensive knowledge." (Hugh Williamson was just that and, as befitted a Pennsylvania-born, but Edinburgh-trained, physician, he was later to be a founder of the New York Literary and Philosophical

Society.) Henry went on to mention "Dr Franklin, who . . . will carry you all the philosophical news," a new publication of Dr Percival which "he purposes to send by the first opportunity to you and Dr Morgan" and "Dr Priestley (who) is proceeding in his experiments and intends in about two months to publish . . . (details of how) he procures air six times more salubrious than common air (that is, the landmark discovery of oxygen)." Finally, it was reported that—in accord with best network practice—"Dr Priestley has kindly put up my name at the Royal Society; he and some other of my friends had recommended me to Dr Franklin who had promised me all his interest".

This letter, which also contains the remarks about "the unhappy divisions that prevail between the mother country and her colonies" shows this one strand of the network at the height of its power, at the moment when war was about to disrupt its activities and transform its contexts. Even so, the next letter from Henry to Rush (dated May 10, 1784) remarks that "the progress of literature and philosophy have not been wholly interrupted. As a proof of this a Society for the promotion of these objects has been established in this place, and has obtained some degree of celebrity . . . You were once so obliging to offer to get me elected into your respectable Society; and with your permission I will propose your name to be enrolled among our Honorary Associates." Henry's letter was conveyed by "Mr Joseph Hadfield" (who) belongs to a respectable commercial house here . . . and is going to settle in America." Symbolically covering the distance between both Manchester and Philadelphia philosophers and the Royal Society of London, the letter also reports how "a most excellent mathematician and philosopher and a very worthy man from Manchester had been 'black balled', Sir Joseph (Banks) making a party against him". In like manner, Percival wrote to Franklin how "the loss of Sir John Pringle has almost ruined the Royal Society. But you are no stranger, I presume, to the disgraceful contentions of that learned body, and to the conduct of the President." Once again distance from London was but the obverse of the coin of network linkages. Percival also wrote to Franklin saying the Manchester Society was publishing one of his scientific papers and that "I am commissioned to return the thanks of the Society to you for this communication; to request your future correspondence; and to acquaint you that we have honoured our institution by electing you an extraordinary member." Once more, the message was conveyed by a commercial traveller between the two cities. That same envoy also carried Rush's diploma and another letter containing news of Priestley's work and greetings to Mancunians settled in Philadelphia.

Not altogether surprisingly, the American Philosophical Society returned the honour done two of its members by Percival and Henry. The ties of sentiment and interest were further strengthened by the formal exchange of publications. As president of the Manchester Society Percival wrote to Franklin, President of the American Philosophical Society, acknowledging receipt of "the Vol. of American Phil. Transactions" and sending the "present of our Memoirs". His letter referred to a visit of Dr and Mrs Priestley—the doctor "at this time gallantly engaged in attempts to convert the Jews to Christianity"—and touched extensively on the need to find means to purify the air of manufacturing towns, seeking Franklin's advice. We cannot pursue the means by which two other members of the Manchester group were elected to the American Philosophical Society in 1789. Nor can we fully share the surprise with which Percival reported to Rush the following year that "The Academy of Arts and Sciences at Cambridge in New England have lately conferred on me the unsolicited honour of election into their very respectable Society." Nor is it possible to follow the sequence from the French Revolution to the War of 1812 which brought this

particular Anglo-American link to an end. Instead, I shall point to two passages which so perfectly express the common hopes of the members of the Manchester-Philadelphia strand in the network of provincial dissent, that it seems appropriate to conclude by quoting them in full. In October 1786 Percival wrote to Franklin that:

"The diffusion of the arts and sciences through so many extensive regions of the globe must afford a subject of contemplation peculiarly satisfactory to your mind . . . And I hope that Sun which has so long blessed the nations (namely, Franklin himself) will not set till the interests of truth and knowledge, of civil and religious liberty, are truly established in the Western Hemisphere, which it now enlightens".

In March 1790 he put it this way, to Benjamin Rush:

"I honour your enterprising genius, which is continually exerting itself in some liberal scientific and public spirited undertaking. Mental energy seems now to be the characteristic of America; and great are the expectations which may reasonably be formed concerning your rising republic".

I thank the John Simon Guggenheim Memorial Foundation and the National Science Foundation for partial support of the research reported here. I am also indebted to the Trustees of the British Museum, to the Birmingham Public Library, to the Library of the American Philosophical Society and to the Historical Society of Pennsylvania for permission to quote from letters in their possession. Robert Bud, David Miller and other students and colleagues at the University of Pennsylvania provided thoughtful criticism and generous assistance.

The Viking Mission search for life on Mars

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On each of two unmanned Viking spacecraft, a number of investigations are planned to be conducted on the surface of Mars this summer. Included in the science payload are instruments designed to determine the state of chemical evolution, including the possible presence of living organisms, on that planet.

ON July 4, 1976, the first of a pair of unmanned spacecraft is scheduled to touch down on the planet Mars in an area located at 19.5°N and 34°W, in a region of low elevation, which seems to be a drainage basin for a large portion of the equatorial region of Mars (Fig. 1). Two months later, the second Viking spacecraft should touch down further north (44.3°N and 10°W) in another region of low elevation, relatively close to the edge of the northern winter ice cap, and at a latitude where maximum quantities of water vapour have been measured in the Martian atmosphere¹.

Aboard both of these landers will be identical science 'payloads', including cameras, meteorological and seismic stations, and analytical equipment to conduct analyses of the surface material. These investigations are now planned to be conducted over a period of two months on each lander, but it is possible that the experiments will continue over considerably extended periods of time—approaching a full Martian year—if further funding can be made available for an extended Viking mission. The scientific objectives of this mission are manifold, but, among these, an assessment of the organic evolutionary state of the planet including a search for extraterrestrial life, is of paramount importance.

For the first time, another planet is to be probed directly for evidence supportive of modern ideas on the origin of life². Mars, although not a hospitable planet as judged by terrestrial standards³, is the one planet, other than Earth, that most nearly satisfies our theoretical presumptions of the conditions for life to have evolved⁴.

For many years, telescopic evidence of morphological features, and their temporal changes, inspired speculative controversies about the presence of life on Mars. These older controversies no longer bear discussion in the light of the Mariner Mars-orbiter and fly-by missions (1964–71) which gave us a complete photographic mapping at resolutions of 1 km or better. It is now clear that water in a form available to living organisms is a likely limiting factor. The polar caps seems to contain substantial quantities of ice, but at temperatures far below those that would support any active terrestrial life. The temperate latitudes have congenial daytime temperatures, but humidities far below those known for any earthly desert. A number of mechanisms have, however, been proposed for possible local anomalies that could furnish moisture at temperatures consistent with metabolism⁵. In addition, the Mariner photographs show many surface features that are most easily explained by assuming that episodes of weather, precipitation and the carving of river valleys have occurred in the geological history of Mars.

These considerations have been taken into account, together with the engineering constraints, in planning the selection of landing sites. However, until our first successful landing, we have no reliable empirical basis for predictions about the local habitats of Mars. We have then been obliged

VIKING LANDING SITES

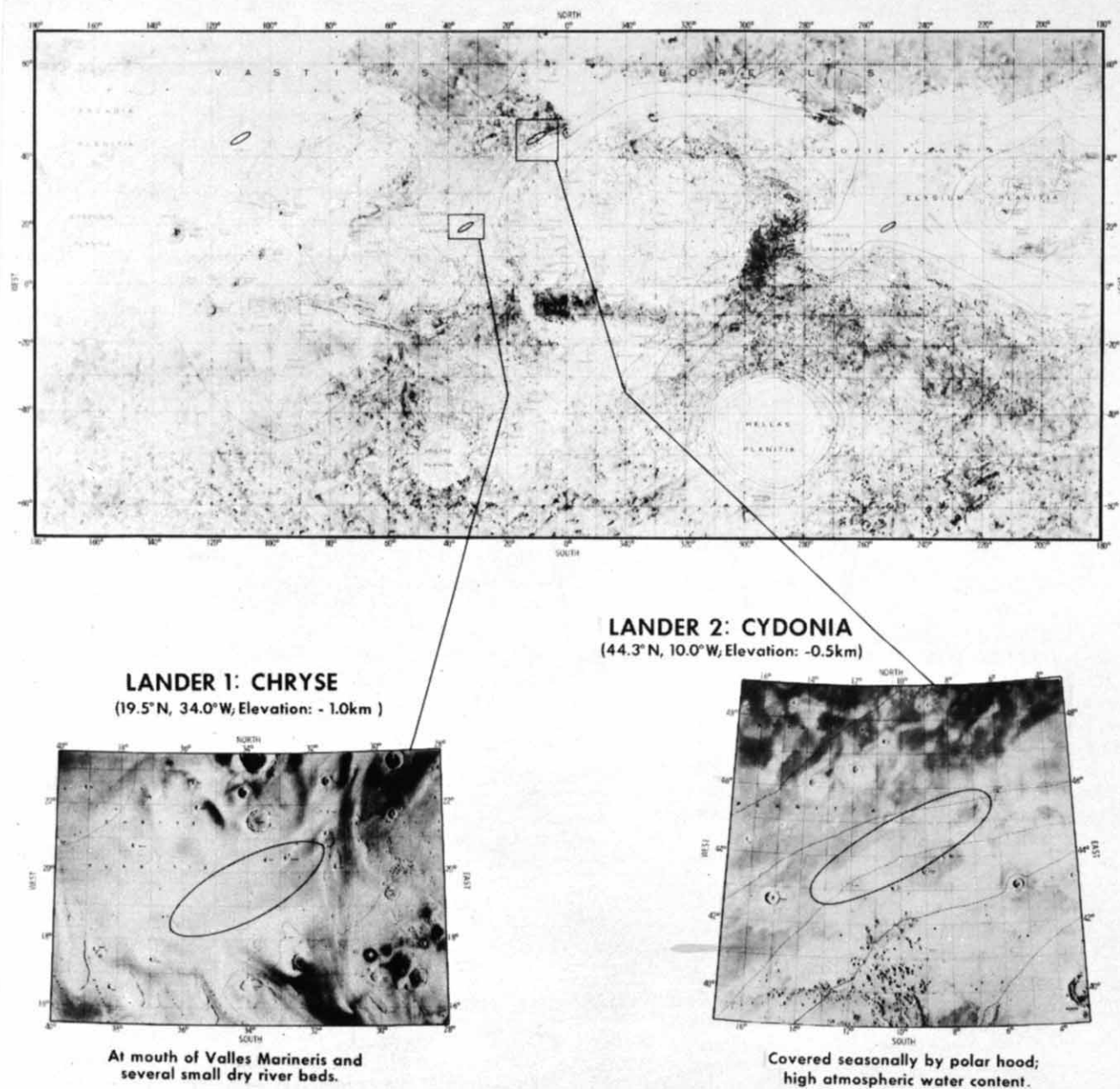


Fig. 1 Map of Mars, constructed from Mariner 9 photographs, showing the two prime landing sites for the Viking landers.

to try to outguess Nature over a range of possible realities in planning these experiments.

Scientific payloads of Viking mission

While all of the experiments are expected to contribute at least peripheral information pertaining to the local milieu of the landers (for example, local temperatures, wind speed and direction, atmospheric composition, elemental composition and magnetic properties of the surface material), three of the investigations hold the greatest promise for contributing directly to the question of organic chemical evolution on Mars.

The first of these is the imaging investigation, which will utilise two facsimile cameras, capable of stereoscopic imaging, on each lander. These cameras will scan the landing site area in black and white, colour and infrared, with a resolution of about 2 mm immediately below the landers. Examination of the surrounding terrain may reveal evidence of past or present living systems. Furthermore, frequent scans of this area may show signs (for example, colour changes) suggestive of living processes⁶.

Also on board each Viking lander is an instrument that will perform organic analyses of the Martian surface material. This is essential not only for obtaining results which may be relevant to Martian life, but also may provide valuable information in assessing the state of molecular complexity if the planet is in a prebiotic state of evolution. The heart of this instrumentation is a mass spectrometer, chosen because of its high sensitivity, wide dynamic range, and broad applicability. For the analyses, samples will be heated in ovens at 200 °C and at 500 °C, and the evolved organic vapour (if any) will first pass through a chromatographic column for separation. A complete gas chromatogram takes 84 min and, during this period, the mass spectrometer will record a complete mass spectrum from mass 12 to mass 200 every 10.3 s (ref. 7).

The biological payload supports three separate experiments which are based on different assumptions about the nature of a possible Martian biology. In the face of our relative ignorance of the local environmental conditions on Mars—for example, up to the present, there is no positive indication that nitrogen is present on Mars⁸—there is ample

reason to approach the question of life detection on Mars with a broad approach. The combination of biological experiments on Viking is thus designed to create a diverse set of environments within which to elicit evidence of metabolism. Three kinds of metabolic question will be asked. Is there evidence for building up of molecules containing carbon starting with ^{14}CO or $^{14}\text{CO}_2$? Can small radioactive organic molecules be broken down to $^{14}\text{CO}_2$? Are gases other than CO_2 produced or consumed? These broad experiments try to measure anabolic and catabolic reactions on Mars. The conditions vary from dry to wet; include the presence of simple and complex nutrient sources, as well as the absence of added nutrients; and include incubation in the light or dark.

For the biological experiments samples will be analysed several times during each mission. They will be obtained from the upper 4 cm of surface material and will be incubated for different periods of time in each experiment. Incubation temperatures are expected to run at $15 \pm 10^\circ\text{C}$. Furthermore, each of the experiments is provided with the capability to perform a control experiment with samples preheated to 160°C for 3 h, in the event that any of the experiments gives a presumptive positive result.

Surface samples (approximately 6 cm^3) will be delivered into the biology instrument by an extendable boom at the end of which a jaw-like scoop digs into the surface to acquire samples (Fig. 2).

The biology instrument contains four modules, one for each of the three experiments, as well as a common service module, which contains a helium source used to rotate incubation cells, purge incubation chambers, and for other operations as well as a system of vents for gases and for liquid wastes. A detailed description⁸ of the instrument is beyond the scope of this communication, but a brief account follows.

The pyrolytic release (or carbon assimilation) experiment is designed to measure either photosynthetic or dark fixation of CO_2 or CO into organic compounds^{10,11}. Both of these gases are known to be present in the atmosphere of Mars, and the experiment is designed to simulate, as closely as conditions permit, the actual Martian environment. The experiment hardware includes a xenon arc lamp with a spectral range and distribution quite similar to the solar spectrum, and with an intensity of about 20% of the maximum Martian solar irradiation at the surface. The light source is provided with a filter to remove ultraviolet radiation below 340 nm to prevent the non-biological fixation of carbon monoxide into organic compounds, as has been reported by Hubbard *et al.*^{12,13}. For this experiment, $20\text{ }\mu\text{Ci}$ of a mixture of $^{14}\text{CO}_2$ and ^{14}CO (in a ratio of approximately

95:5) is injected into the headspace (containing Mars atmosphere) above a 0.25-cm^3 surface sample that has been sealed off in one of the three incubation chambers provided. Incubation proceeds for the next five days in the light. While the incubation is proceeding, a long background count is taken. Following this period, the original incubation atmosphere is automatically vented, and the sample is pyrolysed by heating to 625°C . During this stage of the experiment sequence, any residual initial radioactive CO or CO_2 will be driven out of the sample, through a column, the organic vapour trap (OVT), and into a ^{14}C detector, while organic fragments will be adsorbed on the OVT. After counting at this point in the sequence, the radioactive gases in the detector are automatically vented, and the detector is then heated to remove adsorbed gases. Following this step, and several additional helium purges of the OVT and the detector, the organic matter that had been trapped on the OVT is now eluted by heating it to 650°C for 3 min. During this heating, any trapped organics are released from the column and are simultaneously oxidised to CO_2 by copper oxide present in the packing of the OVT. The effluent from this procedure is purged into the detector and counted. A radioactive peak at this time would constitute presumptive evidence for biological activity, to be followed by a control experiment using heat-sterilised soil.

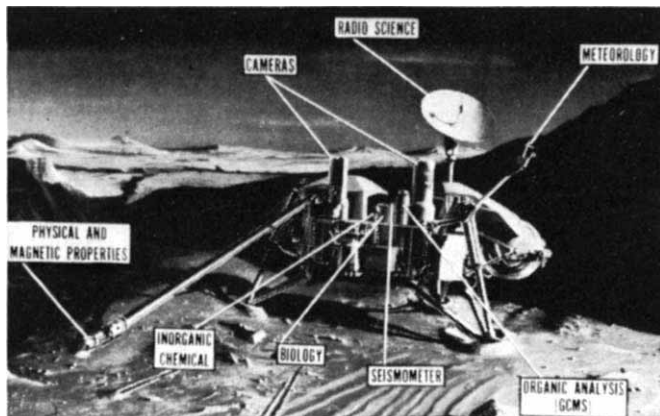
In addition to the sequence just described, the experiment can be modified by the introduction of water vapour into the incubation atmosphere by commands from Earth. Another commandable option in this experiment is to turn off the lamp to conduct a dark incubation.

The labelled release experiment¹⁴ will test for metabolic activity during incubation of a surface sample that has been moistened with a solution of ^{14}C -labelled simple organic compounds, consisting of a mixture of one-, two-, and three-carbon compounds, all uniformly labelled and in dilute aqueous solution. For this experiment, one of the four incubation cells provided in this module will receive 0.5 cm^3 of surface material, and will be sealed with Martian atmosphere trapped in the headspace above the sample. Following this, a background count, lasting approximately 24 h, is taken, after which 0.115 cm^3 of nutrient is automatically injected onto the sample. Incubation proceeds for the ensuing 8 d. A second injection of labelled nutrient then occurs, and incubation continues for 2 d. During the entire time, the atmosphere above the sample is monitored by a ^{14}C detector to determine the kinetics of released labelled gas following each injection. Incubation is ended by a termination sequence to bring the background count down to pre-incubation levels. Following this 'clean-up' operation, the experiment is ready for another analysis cycle.

The gas exchange experiment¹⁵ will be conducted in two different modes using the same equipment. In one mode (the 'humid' mode), Martian surface samples will be incubated in the presence of CO_2 and water vapour. This mode is based on the assumption that substrates may not be limiting on Mars, but that biological activity is dormant in these samples until enough water becomes available in the environment. The second mode (the 'heterotrophic' mode) assumes the presence of frankly heterotrophic organisms in the Martian surface.

For both of these experimental modes, it is further assumed that metabolism of active organisms in the samples will cause the disappearance or release of one or more gases known to be involved in terrestrial metabolic processes. For an analysis, 1 cm^3 of sample is delivered into the single incubation cell. A mixture of CO_2 and Kr (in He) is then added to serve as the incubation atmosphere. After this step, 0.5 cm^3 of nutrient is delivered to the bottom of the incubation cell. During incubation, the sample is retained in an inner-cup within the incubation cell so that the nutrient does not come into direct contact with the sample. The inner cup is porous and allows equilibration between

Fig. 2 Viking lander. Surface sampler is seen extended at left. Biology sample processor and distribution assembly is seen midway between the two cameras. Biology instrument is beneath this component and not visible in this figure.



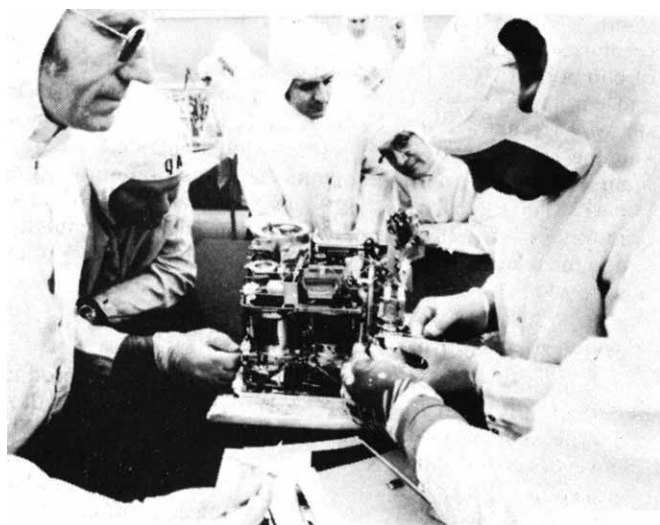
the sample and the incubation atmosphere. Gas samples (100 μ l) are taken from the headspace above the sample immediately after the nutrient has been injected and at specified times thereafter for the next 7 d. The gas samples are swept through a chromatographic column and the components detected by a thermal conductivity detector. If no command to the contrary is given after 7 d in the 'humid' mode, the instrument will automatically inject additional nutrient bringing the total aqueous volume now to 2.5 cm³. With this amount of nutrient, the sample will be wet by the nutrient and incubation now will proceed in the 'heterotrophic' mode. (The nutrient solution contains both D- and L-amino acids as well as other organic substrates, growth factors and inorganic compounds.) Incubation continues in this mode with gas samplings at stipulated intervals for the next 9 d. At this point, options exist to proceed in various directions, depending on the data obtained. One is to drain the nutrient from the bottom of the cell and to introduce fresh atmosphere and fresh nutrient, and to incubate the original sample for an additional 19 d. Or, the initial incubation conditions can simply be extended for an additional 19-d period (that is, without draining the original nutrient or changing the gas atmosphere). If circumstances warrant it, the nutrient can be drained, the original sample dried, and a new sample introduced on top of the first one for a second analysis in the 'heterotrophic' mode.

In addition to the ¹⁴C data from the two radioactivity detectors, and the gas chromatographic data, several other measurements will be telemetered periodically from the spacecraft during the course of these experiments. These include the status of the xenon lamp, the position of each incubation cell, temperature measurements of each incubation cell being used, and temperature measurements of both nuclear detectors, of the OVT and of the gas chromatographic column and detector. In addition, we will receive data on the pressure of the critical helium reservoir in the common services module.

Figure 3 shows one of the flight instruments in its final stages of assembly. It contains, in addition to the incubation cells and sample distribution system, four different gas supplies, two different nutrients, two ¹⁴C detectors, a thermal conductivity detector and chromatographic system, 43 different heaters, 4 thermoelectric coolers, 39 miniature latching solenoid valves, a xenon arc lamp, 22,000 transistors, and 18,000 other electronic parts. All of this is packaged into a volume somewhat greater than 1 foot³, and weighs 35 pounds.

The three biology experiments differ in their sensitivity, as measured by their responses to terrestrial organisms.

Fig. 3 Biology flight instrument in final stages of assembly.



Both the gas exchange and labelled release experiments have, by now, been tested against a wide variety of soils and they readily "detect" the presence of microbial flora in these samples when they are incubated in ambient terrestrial conditions. When these soil samples are incubated under the conditions in which the experiments will be operated on Mars, the responses are delayed by prolonged lag periods, but qualitatively similar responses are seen. In contrast, the pyrolytic release experiment does not respond to terrestrial soils at all when incubated in "Martian" conditions. Only when water is included in the incubation test cell is terrestrial biological activity demonstrable in this experiment. Accordingly, during the long test programme that preceded the manufacture of the flight instruments, several 'standard' soils were used to challenge the two 'wet' experiments. For the pyrolytic release experiment, soil was preincubated in the light in the presence of water to provide fixed radioactive carbon compounds, dried, and then used in this form to test the equipment. Since the final flight hardware is designed to work on Mars, and not on Earth, and because the design of the biology package precludes repetitive incubations (beyond four per experiment), and because of the rigid requirements to prevent terrestrial contamination, the flight instruments were not, and could not be, tested with terrestrial soils. Precursor instruments, built to flight specifications (with minor exceptions), did go through at least one prolonged test in simulated Martian conditions using one of our 'standard' soils, (containing 1×10^6 aerobic, and 5×10^4 anaerobic organisms per g; with a pH of 4.8 and an organic content of 0.8%). In addition, a flight-like instrument was installed on a lander and tested as part of a complete system, but these tests did not include analysis of any soils. Rather, known mixtures of gases were processed through the gas exchange hardware, and ¹⁴CO₂ was used to challenge the other two experiments. These tests¹⁶, while not completely satisfying, in that only a part of the complete system was tested, gave results entirely consistent with extensive prior testing at the module level. Finally, at the component level (that is, detectors, chromatograph columns, and so on), many of the actual flight components were extensively tested for their scientific as well as their engineering characteristics. In these tests, no significant anomalies were uncovered. In summary, the testing programme was less than optimal, being limited by time and fiscal constraints, but met the minimum standards for confidence that the instruments will perform their nominal functions after landing on Mars.

The team of scientists selected by NASA, in 1969, to plan and conduct the biological investigation on the Viking spacecraft consisted of the authors and the late Dr Wolf Vishniac (University of Rochester, Rochester, New York). The following individuals are collaborators in these investigations: Bonnie Berdahl, Glenn Carle and Richard D. Johnson (NASA, Ames Research Center, Mountain View, California); George Hobby (California Institute of Technology, Pasadena, California); Jerry Hubbard (Georgia Institute of Technology, Atlanta, Georgia); and Patricia Straat (Biospherics, Incorporated, Rockville, Maryland).

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articles

Xenon record of the early Solar System

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We suggest that xenon isotopes in meteorites and in outer regions of the Sun contain debris from a supernova which exploded in the vicinity of the present Solar System, and we conclude that xenon in the outer planets is enriched in this supernova debris.

In 1960 Reynolds¹ noted that the isotopic composition of xenon trapped in the Richardton olivine-bronzite chondrite and in the Murray carbonaceous chondrite differed in a uniform manner from that of xenon in air. He concluded that the general isotopic anomaly pattern of xenon in meteorites was primordial in origin, that is, characteristic of xenon incorporated in the meteorites at the time of their formation. Subsequent analyses confirmed that xenon trapped in other stone meteorites exhibits a similar pattern of isotopic anomalies²⁻⁴, now commonly designated as AVCC xenon.

In 1964 Reynolds and Turner⁵ analysed the isotopic composition of xenon released by stepwise heating of the Renazzo chondrite and observed an unusual isotopic anomaly pattern in the xenon released at 700–900 °C. Relative to the two more abundant, β -shielded isotopes, ^{128}Xe and ^{130}Xe , there was an enrichment of the heavy isotopes, $^{131-136}\text{Xe}$, of the general form expected for a fission spectrum. It was noted that ^{124}Xe and ^{126}Xe also seemed to be enriched in the xenon released at these temperatures, and small amounts of these isotopes would have to be explained by some additional process. During the next eight years, analyses in several laboratories of xenon released by stepwise heating of other carbonaceous chondrites confirmed the presence of this strange xenon component⁶⁻¹². Attempts to understand the origin of this component were, however, focused exclusively on the enrichment of the heavy isotopes, and the strange xenon came to be referred to as “fission-produced xenon”⁹, “Renazzo fission”⁸ and “CCF” as an abbreviation for carbonaceous chondrite fission¹³.

Possible explanation

In discussing the anomalous xenon in Renazzo, Reynolds and Turner⁵ had stated that there were relatively large errors in the measurements of the light isotopes, but by 1972 relatively large enrichments of this anomalous xenon had been observed in other carbonaceous chondrites. It was then noted¹⁴ that all available data on xenon released from carbonaceous near 800 °C showed an excess of the light, proton-rich isotopes in addition to the enrichment of heavy, neutron-rich isotopes. This observation could not be explained by any of the mechanisms previously proposed to account for the excess heavy xenon isotopes—spontaneous fission⁵ of ^{244}Pu , a carrier⁸ of heavy xenon adsorbed on carbonaceous material, neutron-induced fission⁹, isotopic mass-fractionation^{10,11}, or fission¹⁵⁻¹⁷

of superheavy elements. We therefore suggested that the anomalous xenon represents an isotopically distinct component which had been trapped in carbonaceous chondrites and suggested two possible mechanisms for its production before being incorporated into the meteorite; either by a mixture of severely mass fractionated xenon with excess $^{131-136}\text{Xe}$ from neutron-induced fission of transbismuth nuclides, or by a supernova explosion generating an excess of the proton-rich isotopes via the p process and an excess of the neutron-rich isotopes via the r process. At that time we noted that there was, however, no evidence among the other elements for distinct isotopic components from separate nucleosynthesis events. But isotopically distinct components of oxygen¹⁸ and magnesium¹⁹ were found in carbonaceous chondrites within the next two years, and others²⁰⁻²² expanded on the possible role of a supernova explosion in producing the anomalous xenon in carbonaceous chondrites.

Since the term CCF xenon had connotations of a mechanism which could only account for part of the isotopic pattern of anomalous xenon released from carbonaceous chondrites near 800 °C, we suggested component X as a more innocuous term for the xenon component enriched in the light and heavy isotopes¹⁴. In 1974 we²³ reported that component X contained at least seven of the nine stable xenon isotopes and noted that AVCC xenon itself contained component X, that is, before the incorporation of xenon in stone meteorites there were sufficient quantities of component X to produce an observable alteration in the isotopic composition of xenon in the region of accretion.

Very recently Lewis *et al.*²⁴ reported the results of a beautiful detailed study on the abundance and isotopic composition of noble gases in separated mineral fractions of the Allende carbonaceous chondrite. Their results offer the best description of component X. In the following sections we will use these²⁴ and earlier results of studies on carbonaceous chondrites by stepwise gas extraction data from studies on terrestrial xenon, and the results of studies on solar-wind implanted xenon in lunar soil to determine the quantity and distribution of component X in the solar system. This information will then be used to seek a better understanding of the origin of component X and the record it contains of events in the early history of the Solar System.

Terrestrial xenon

It is now known that radiogenic ^{129}Xe , the decay product of extinct ^{129}I which Reynolds²⁵ discovered in the Richardton chondrite, is a common component of meteoritic xenon. The discovery of radiogenic ^{129}Xe in mantle-derived CO_2 (refs 26–28) demonstrates that the Earth started to retain this decay product at about the same time as the meteorites. Because of uncertainties in correcting for this radiogenic component, we will not

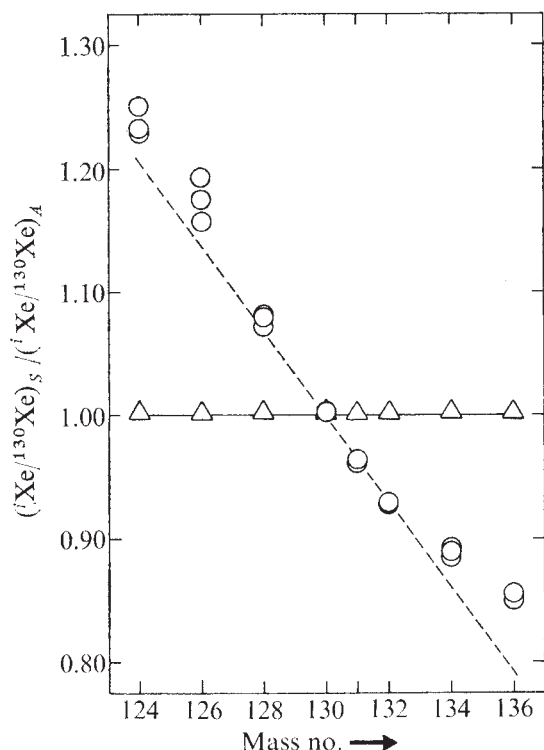


Fig. 1 A comparison of xenon isotope ratios in solar (S) and terrestrial xenon (A). The latter has been corrected for fission products from ^{244}Pu . Three separate estimates of solar xenon are in close agreement, and two of the estimates are coincident for xenon isotope ratios at masses 131 (refs 47, 48), 132 (refs 47, 48) and 136 (refs 43, 47).

consider the abundance of ^{139}Xe in comparing terrestrial and extraterrestrial xenon. Since excess $^{131-136}\text{Xe}$ constitutes a major part of component X, however, it will be necessary to correct for fissionogenic $^{131-136}\text{Xe}$ from another extinct radionuclide, ^{244}Pu . The latter was discovered in the Pasamonte achondrite by Rowe and Kuroda²⁹, and should have produced more than 15 times as much fissionogenic $^{131-136}\text{Xe}$ as the spontaneous fission of ^{238}U over the history of the Earth.

The results of several analyses³⁰⁻³² of noble gases in shales and other carbonaceous minerals reveal extremely high concentrations of xenon, apparently from physical adsorption³³. The suggestion that the atmosphere may contain only one-tenth³¹ of the Earth's total inventory of xenon has been confirmed by analyses²⁸ of noble gases in a volcanic xenolith from the mantle. We will therefore consider the contribution from the fission of ^{244}Pu to the total concentrations of $^{131-136}\text{Xe}$ in the 'crust-atmosphere' system, accepting the decay product of 17 Myr ^{129}I in the Earth's mantle²⁸ as evidence that ^{244}Pu in the mantle has made a negligible contribution to the isotopes, $^{131-136}\text{Xe}$, in this system. Since there are 8.2×10^{35} atoms of ^{136}Xe in the atmosphere³⁴, we estimate that the Earth contains a total of 8.2×10^{36} atoms of ^{136}Xe . Podosek³⁵ has shown that $^{244}\text{Pu}/^{238}\text{U} = 1.27 \times 10^{-2}$ when the St Severin meteorite started to retain ^{136}Xe from the decay of ^{244}Pu . Assuming that the Earth started retaining fissionogenic ^{136}Xe , ^{136f}Xe , at this time, an average uranium content of 1.3 p.p.m. for the Earth's crust³⁶, and a 6% fission yield at ^{136}Xe , then the decay parameters of ^{244}Pu (ref. 37) indicate that terrestrial xenon contains isotopes in the ratio $^{136f}\text{Xe}/^{136}\text{Xe} = 0.018$. From the fission yields of ^{244}Pu (ref. 38), and the isotopic composition of atmospheric fission-free terrestrial xenon: $^{124}\text{Xe}:^{126}\text{Xe}:^{128}\text{Xe}:^{130}\text{Xe}:^{131}\text{Xe}:^{132}\text{Xe}:^{134}\text{Xe}:^{136}\text{Xe} = 2.353:2.206:47.03:100:518.1:655.7:252.3:213.5$. We will use the symbol 'A' to designate fission-corrected atmospheric xenon of this composition in the following discussion on solar and meteoritic xenon. It should be stressed that we have purposely attempted

to make a conservative estimate of the contribution of ^{244}Pu to $^{131-136}\text{Xe}$ by selecting one of the higher estimates for the total xenon content of the Earth and by assuming that all of this xenon is in the 'crust-atmosphere' system. This was done so that we will not overestimate the amount of component X in other parts of the Solar System.

Solar xenon

Marti⁴⁰ first identified the presence of solar-wind implanted xenon in the Pesyanoe meteorite. Later studies of xenon in lunar fines and breccias confirmed the general isotope pattern which Marti reported in solar xenon. Several lunar scientists⁴¹⁻⁴⁴ interpreted the results of their xenon analyses as evidence that the isotopic compositions of atmospheric and solar xenon are related by mass fractionation and attempted to explain AVCC xenon as a mixture of solar xenon with CCF xenon. Although this interpretation of the three known reservoirs of xenon is not consistent with the experimental data^{23,45}, it was widely accepted as additional evidence for a large component of fission-produced xenon in carbonaceous chondrites.

Bogard *et al.*⁴⁶ and others⁴⁵ have noted that the 'true' isotopic composition of solar xenon is still not known because many variations observed in the isotopic composition of xenon in lunar soils arise from mass fractionation. To compare the isotopic composition of terrestrial and solar xenon, we have therefore selected similar estimates of the latter from analyses of xenon in lunar fines by three, well recognised laboratories: Pepin's laboratory at the University of Minnesota⁴³, Wasserburg's laboratory at the California Institute of Technology⁴⁷, and Geiss' laboratory at the University of Bern⁴⁸. The authors identify this xenon as trapped lunar Xe (ref. 43), SUCOR Xe (ref. 47), and BEOC 12001 Xe (ref. 48). We normalise these xenon spectra to ^{130}Xe to facilitate identification of component X, which is enriched in both the light and the heavy isotopes relative to ^{130}Xe (refs 14 and 23).

The relationship between isotope ratios in solar xenon, $(^{130}\text{Xe}/^{136}\text{Xe})_S$, and xenon isotope ratios in fission-free atmospheric xenon, $(^{130}\text{Xe}/^{136}\text{Xe})_A$ is shown in Fig. 1. The dashed line corresponds to 3.45% per a.m.u. fractionation of the $(^{130}\text{Xe}/^{136}\text{Xe})_A$ values. This fractionated terrestrial xenon is very similar to solar xenon at $^{128-132}\text{Xe}$, but the agreement is not very good at ^{124}Xe , ^{126}Xe , ^{134}Xe and ^{136}Xe . Since studies of xenon in carbonaceous chondrites show that component X is highly enriched in these four isotopes, we interpret Fig. 1 as evidence that solar-type xenon contains a larger fraction of component X than does terrestrial xenon. Deviations of the heavy isotopes from the fractionation line in Fig. 1 suggests that ~7% of ^{136}Xe in solar-wind xenon is from component X. This is a lower limit on the amount of component X in solar xenon; higher values would result if terrestrial xenon contains component X or a larger component of fissionogenic $^{131-136}\text{Xe}$ than indicated by our purposely conservative estimate.

Meteoritic xenon

The isotopic compositions reported²⁴ for xenon in separated mineral fractions of Allende confirm the presence of an enrichment of both light and heavy xenon isotopes in component X. Figure 2 shows the correlation between the enrichment of the two lightest xenon isotopes and the heaviest xenon isotope in mineral separates of Allende²⁴ and in the gas released near 800 °C from earlier stepwise heating experiments on carbonaceous chondrites^{5,8,10-12}. Our estimate of fission-free atmospheric xenon is also shown in Fig. 2, and changes in its composition which can be accounted for by mass fractionation are represented by dashed lines. We will discuss the intersections of the fractionation lines with the solid correlation lines later, after considering correlations of other constituents in the separated mineral fractions of Allende.

It has been suggested²⁴ that we were too hasty in concluding that the correlation shown in Fig. 2 is evidence that excesses of the light and heavy xenon isotopes were mixed before being

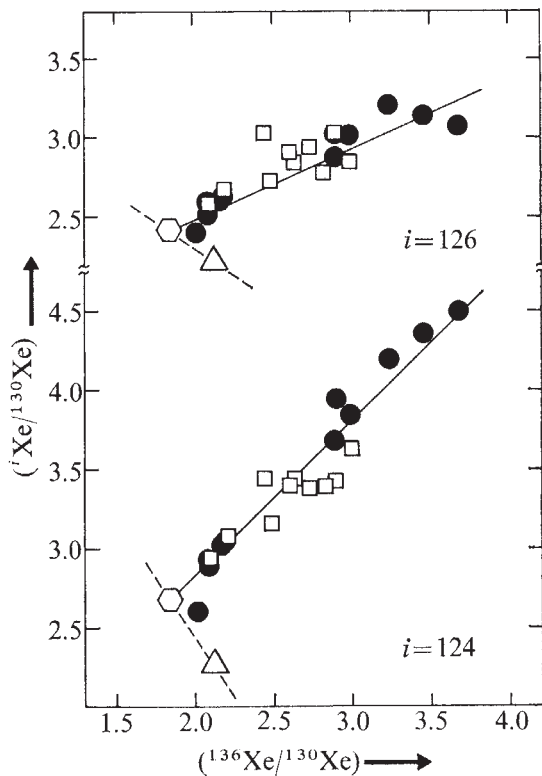


Fig. 2 The correlation of excess ^{124}Xe , ^{126}Xe and ^{136}Xe in mineral fractions of Allende²⁴ (●) and in xenon released by stepwise heating (□) carbonaceous chondrites^{5, 8, 10–12}. Isotope data from stepwise heating experiments are from a tabulation by Sabu *et al.*²³. Fission of a volatile superheavy element cannot account for the correlations observed between light and heavy xenon isotopes.

trapped in meteorites. Anders *et al.*⁴⁹ suggest that the excess heavy xenon isotopes may be from *in situ* decay of a volatile superheavy element and the excess light xenon isotopes may be from an unknown isotopic fractionation mechanism which occurred during trapping. We will briefly examine the experimental evidence for this model.

First, we will examine the correlations of excess ^{136}Xe with volatile elements in mineral fractions at Allende, since the previously reported correlation¹⁶ of excess ^{136}Xe with ^{132}Xe , In and Hg in bulk meteorite samples seems to form the basis for this model. The correlations which result from the recent analyses^{24, 49} on In and Xe are shown in Fig. 3; Hg values were not reported. In 3Cl the concentrations of excess ^{136}Xe is enriched 180-fold over its concentrations in bulk Allende, but In concentrations are unchanged. The dashed line in Fig. 3 shows the 45° correlation of excess ^{136}Xe with ^{132}Xe in average carbonaceous chondrites¹⁶. The xenon in separated mineral fractions of Allende seem to define three sets of correlations between excess ^{136}Xe and ^{132}Xe , none of which agrees with the correlation found in bulk carbonaceous chondrites. As we have noted earlier⁵⁰, the 45° correlation reported¹⁶ between excess ^{136}Xe and ^{132}Xe in various meteorites occurs because the bulk isotopic composition of xenon in these meteorites is constant. Fractions of xenon enriched in component X can, however, be isolated by stepwise heating experiments or by separating mineral fractions. As the degree of separation of component X increases, the correlation with ^{132}Xe decreases. The correlation is best for bulk meteorite samples because AVCC xenon itself contains component X. But we find little, if any, evidence in Fig. 3 to support the previously reported correlation¹⁶ of excess ^{136}Xe with In or ^{132}Xe .

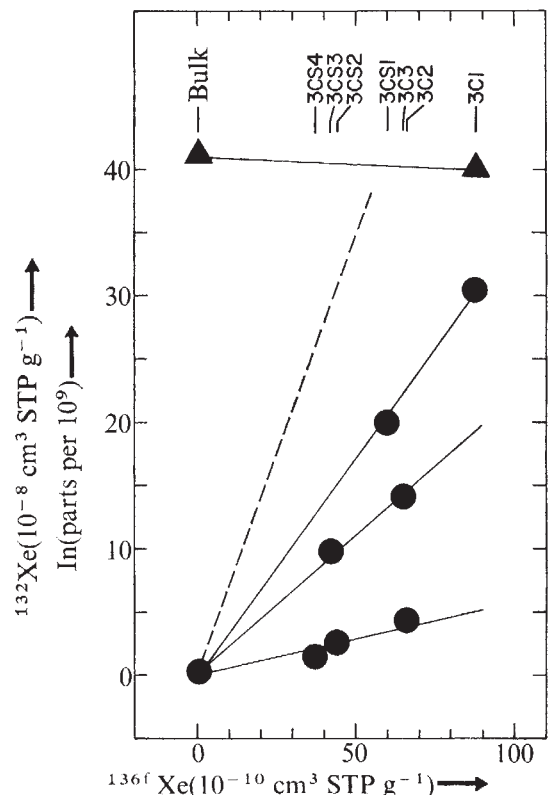
To seek evidence that the excess light xenon isotopes in component X result from an unknown fractionation process, we next look for the effects of this process in the isotopes of krypton. The isotope ratios of krypton and xenon in the

separated mineral fractions of Allende²⁴ are shown in Fig. 4. These isotope ratios show an excellent correlation between the enrichment of the light xenon isotopes and the heavy krypton isotopes. Anders *et al.*⁴⁹ suggest that the heavy krypton isotopes were enriched by fractionation, but we know of no fractionation process which could enrich the heavy isotopes of krypton and the light isotopes of xenon to produce the correlations shown in Fig. 4. We have not considered the light krypton isotopes because of hydrocarbon background and large uncertainties²⁴ but conclude from Fig. 4 that isotopic mass fractionation is not a viable mechanism to account for the excess light xenon isotopes in component X.

We will therefore consider the possibility that component X indeed represents an isotopically distinct component of xenon which has been trapped, together with terrestrial type xenon, in meteorites. Figure 5 compares the isotopic composition of xenon in the Earth, in average carbonaceous chondrites⁴ and in the mineral fraction of Allende showing the most anomalous xenon, 3CS4 (ref. 24). The dashed line defines the values of $(^{124}\text{Xe}/^{130}\text{Xe})_A$ with 2.25% per a.m.u. fractionation. This fractionation is also shown by the hexagons in Fig. 2 at the intersections of the dashed fractionation lines and the solid correlations. AVCC xenon, the xenon in separated mineral fractions of Allende, and the xenon released by stepwise heating of carbonaceous chondrites can be understood in terms of various mixtures of mass fractionated terrestrial xenon and component X. Deviations from the fractionation line at ^{136}Xe suggest that ~7% of the ^{136}Xe in AVCC xenon, and 50% of the ^{136}Xe in the 3CS4 fraction of Allende, are from component X. We regard these values as lower limits for reasons stated earlier.

Before considering the implications of component X, we wish to stress that results of the recent analyses on xenon in mineral separates of Allende²⁴ are inconsistent with our earlier suggestion¹⁴ that the excess light isotopes in component X might result from mass fractionation. In addition to the reasons cited earlier for rejecting this mechanism, Lewis *et al.*²⁴ point

Fig. 3 Correlations of the 'fission' product, ^{136}fXe , with In (▲) and ^{132}Xe (●) in mineral fractions of Allende^{24, 49}. The dashed line represents the correlation of ^{136}fXe with ^{132}Xe in bulk meteorites if the amount of ^{136}fXe is calculated in the same manner used to calculate ^{136}fXe in Allende minerals²⁴.



out that there is a definite excess of ^{124}Xe in the anomalous xenon above any plausible fractionation line through ^{126}Xe , ^{128}Xe and ^{130}Xe . This can be seen in Fig. 5 for the 3CS4 xenon. We therefore conclude that component X represents material from a supernova.

Implications of component X

We have shown that component X, an isotopically distinct type of xenon, is present in the Sun and in meteorites. The isotopic composition of xenon in the Earth, in meteorites and in the Sun can be understood in terms of mixtures of component X, fractionated terrestrial xenon, and radioactive decay processes. Terrestrial xenon seems to contain the least amount of component X, although recent analyses by Lightner and Marti⁵¹ suggest that indigenous lunar xenon also contains little or no component X. We offer the following outline of a model to explain the abundance and distribution of component X, using the term Y when referring to the other xenon component.

Considering first the abundance of X and Y, it should be noted that both components made major contributions to xenon in our Solar System. The relatively small contribution of X to terrestrial and indigenous lunar xenon suggests that Y was dominant in the region where the inner planets accreted. Although X accounts for up to 50% of the ^{136}Xe in some mineral fractions of meteorites, such as the 3CS4 fraction from Allende, these minerals constitute such a small fraction of the total meteorite that they might be attributed to inclusions of small amounts of 'dust grains' which formed outside our Solar System. We have, however, shown that X is responsible for ~ 7% of the ^{136}Xe in both AVCC xenon and solar xenon. This observation requires relatively large amounts of X in the Solar System. In view of this, we regard the dominant role of X in certain meteorite minerals as evidence that X too was dominant in certain regions of our Solar System. We therefore conclude that the supernova was in the immediate vicinity of

Fig. 4 Correlations of excess heavy krypton isotopes with excess light xenon isotopes in mineral fractions of Allende²⁴ (●). The isotopic composition of atmospheric noble gases are represented by the open triangles (△).

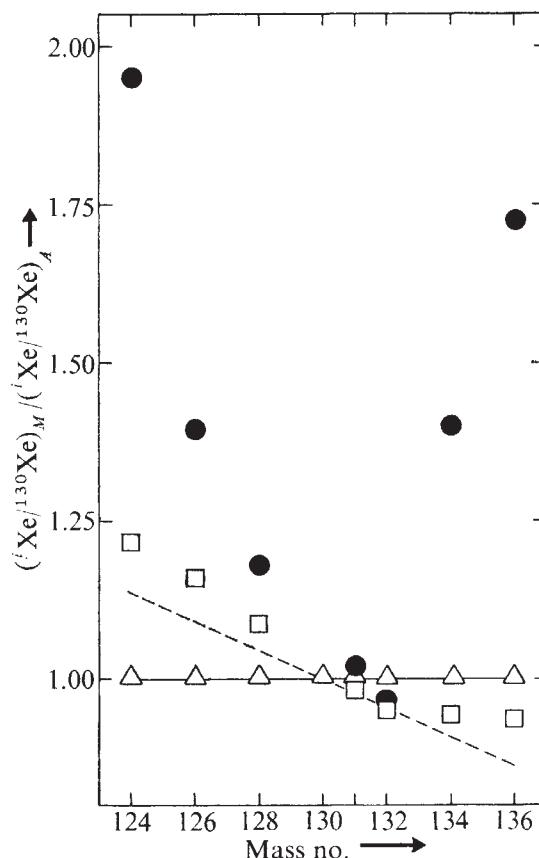
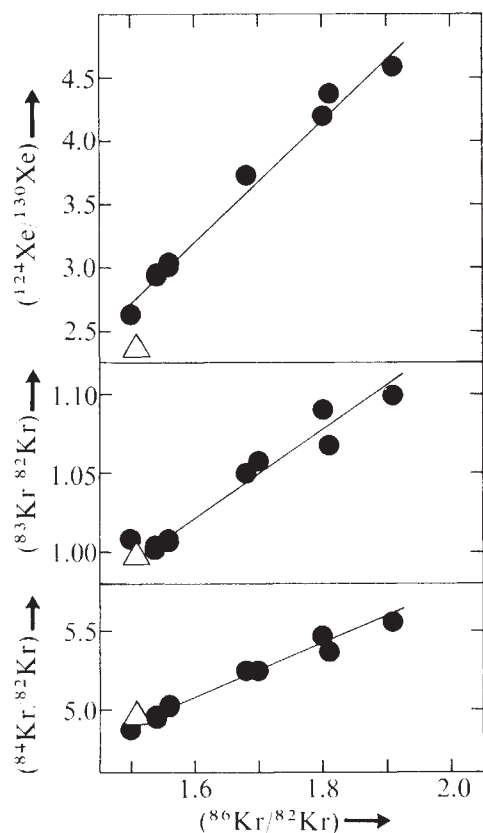


Fig. 5 A comparison of the xenon isotope ratios in AVCC⁴ (□), in the 3CS4 fraction of Allende²⁴ (●) and in fission-free terrestrial xenon (△). Linear fractionation of the latter, 2.5% per a.m.u., is represented by the dashed line. Deviations of meteorite xenon from this line arise from the presence of component X.

our Solar System.

Considering next the distribution of X and Y, we suggest that these two isotopic components may correlate with other properties of our Solar System. As noted above, Y seems to have been dominant for the inner planets. In bulk meteorites X accounts for ~ 7% of the ^{136}Xe , but X is dominant in some meteorite minerals. The physical position of the parent meteorite material in the asteroid belt, and the occurrence of X-rich and Y-rich minerals in meteorites, suggests that this region may represent the boundary between regions where X and Y were dominant. There is an abrupt change in the character of planets in the region of the asteroid belt. The giant, less-dense planets lie beyond the asteroid belt and may contain relatively large contributions from X.

From the above considerations we conclude that our Sun was very likely once the smaller member of a binary star system. A supernova explosion of the other member produced debris rich in component X, in heavy krypton isotopes²⁴, in ^{244}Pu (ref. 52), ^{16}O (ref. 18) and ^{24}Mg (ref. 19), and perhaps rich in ^{26}Mg (ref. 19), helium and neon²⁴. We expect that chemical elements of the outer planets will be enriched in these. Some of the debris also accumulated in the Sun, but the total solar abundance of X is not well defined, since material from the supernova may be enriched in the outer layers of the Sun. Hoyle⁵³ pointed out that initially low concentrations of helium, and high concentrations of metals in the iron group, in the Sun's core may account for the apparently low flux of solar neutrinos⁵⁴.

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Translation *in vitro* of vesicular stomatitis virus mRNA lacking 5'-terminal 7-methylguanosine

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The 5'-terminus 7-methylguanosine of vesicular stomatitis virus mRNA is not essential for translation of the mRNA. The 7-methylguanosine seems to have a mediating rather than an obligatory role in ribosome binding by the mRNA and in mRNA translation.

MESSANGER RNAs (mRNAs) from several animal viruses and animal cells contain the 5'-terminal structure m⁷G^{5'}ppp^{5'}NmpN(m)p¹⁻¹⁰. Vesicular stomatitis virus (VSV) and reovirus mRNAs synthesised by virion polymerases in the absence of S-adenosyl methionine contain the respective 5'-termini G^{5'}ppp^{5'}ApAp and ppGp; these RNAs will not direct synthesis of virus proteins in cell-free extracts from wheat embryo unless the methylated 5'-terminus m⁷G^{5'}ppp^{5'}N(m)pNp has been formed^{11,20}. Also, unmethylated reovirus mRNA will not bind to wheat germ ribosomes¹². In the light of these experiments and others involving the chemical removal of the terminal 7-methylguanosine from globin mRNA¹³, it has been suggested that the 7-methylguanosine is obligatory for translation of all mRNAs in eukaryotic cells¹¹⁻¹³.

We were led to question the requirement for 7-methylguanosine in translation by the finding that the 5'-terminus of poliovirus mRNA purified from the polyribosomes of infected HeLa cells has the 5'-terminus pUp¹⁴. We present here results which show, in contrast to previous work¹¹, that a terminal 7-methylguanosine is not obligatory for translation of VSV mRNAs. Oxidation or removal of the 7-methylguanosine reduces the rate of protein synthesis about threefold and has a similar effect on the rate of mRNA binding to ribosomes in a reticulocyte cell-free system. Furthermore, some of this inhibition may be due to non-specific effects of periodate oxidation.

Removal of 7-methylguanosine

Cells infected with VSV contain five monocistronic viral mRNAs which encode the five viral proteins¹⁵⁻¹⁷, and these mRNAs are complementary to the single-stranded RNA genome of the virus¹⁸. VSV mRNAs purified from the polyribosomes of infected baby hamster kidney cells all possess a common 5'-terminal tetranucleotide structure, m⁷G^{5'}ppp^{5'}mAmpmAmpCp. This has only partial base methylation of the first adenosine and partial base and ribose methylation of the second adenosine⁵. The 5'-terminal 7-methylguanosine, because it has free 2',3' (ribose) hydroxyl groups, is susceptible to oxidation with periodate, yielding a 2',3'-dialdehyde. Further treatment with aniline results in loss of the 7-methylguanosine by β -elimination. We found that the procedure which previously gave quantitative removal of this nucleoside from the terminal tetranucleotide⁵ was unsuitable for use on whole mRNA because it caused extensive degradation and aggregation of the mRNAs as well as some uncharacterised modifications of internal nucleotides. Therefore, we used the procedure detailed in Fig. 1 which involves use of less periodate and removal of periodate from the initial reaction by successive ethanol precipitations of the RNA before treatment with aniline. This procedure minimised but did not eliminate degradation, as is shown by polyacrylamide gel electrophoresis of the denatured mRNA (Fig. 1). The viral protein(s) encoded by the mRNA in each band have been described previously¹⁶ and are indicated in the legend to Fig. 1. Both periodate oxidation and β -elimination resulted in detectable levels of degradation which are most obvious in the decreases in the peak heights of the larger RNA species. It is difficult to quantify the exact amount of degradation of each RNA species because of the presence of multiple RNA species on the gel; however, the results suggest a loss of about 50% of N protein

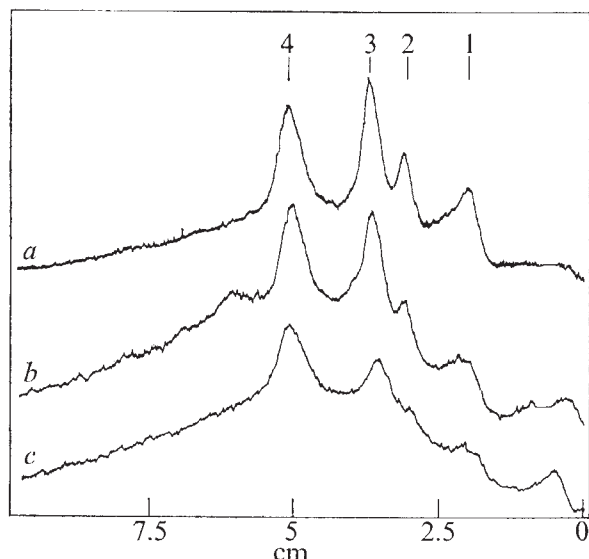


Fig. 1 Polyacrylamide gel electrophoresis of oxidised and β -eliminated VSV mRNAs. ^{32}P -labelled VSV mRNA was isolated by the following modification of a previous procedure¹⁵. A sample (2 l) of BHK-21 cells in spinner culture at 4×10^5 cells per ml were concentrated by centrifugation, washed twice with 100 ml of PO_4 -free medium and then resuspended in 400 ml of PO_4 -free medium. Actinomycin D (final concentration $5 \mu\text{g ml}^{-1}$) and VSV (multiplicity of infection, 10) were then added, and incubation at 37°C continued. One hour after addition of virus, 20 mCi of carrier-free $^{32}\text{PO}_4$ (New England Nuclear) was added, and incubation continued for 3 h. Infected cells were then isolated by centrifugation and resuspended in 10 ml of a solution containing 0.01 M MgCl_2 , 0.01 M Tris-HCl (pH 7.4) and $25 \mu\text{g ml}^{-1}$ DNase I (Worthington, DPF). Cells were lysed by addition of 0.1% sodium dodecyl sulphate (SDS) and incubated at 37°C for 5 min. Then the extract lost detectable viscosity, and SDS and sodium acetate (pH 5.2) were added to final concentrations of 1% and 0.2 M, respectively, followed by extraction with an equal volume of a solution containing equal volumes of redistilled phenol and chloroform. After centrifugation, the aqueous layer was withdrawn and the phenol layer was re-extracted with 5 ml of 0.2 M sodium acetate (pH 5.2). The combined aqueous layers were then precipitated with 2 volumes of ethanol, followed by isolation of VSV mRNA by binding to and elution from 0.3 g of oligo(dT)-cellulose¹⁵. This procedure yielded from 3×10^8 to 10^9 c.p.m. in approximately 300 μg of mRNA. For periodate oxidation, 300 μg of lyophilised VSV mRNA (or 100 μg of VSV mRNA combined with 200 μg of unlabelled f2 phage RNA) was resuspended in 0.2 ml of 10 mM NaIO_4 (freshly dissolved) and incubated for 1 h at 23°C in darkness. The RNA was then precipitated three times by addition of 0.4 ml of 0.1 M sodium acetate (pH 5.2) and 0.8 ml of ethanol. Half of the oxidised RNA was then resuspended in 0.1 ml of a solution containing equal volumes of 0.33 M aniline (pH 4.5, twice vacuum redistilled) and 0.15 M sodium acetate (pH 4.5) followed by incubation at 23°C for 4 h. The RNA was then precipitated with ethanol three times as above. Samples (approximately 10^5 c.p.m. each) of untreated (a), periodate-oxidised (b), and periodate-oxidised, β -eliminated RNA (c) were then denatured and subjected to electrophoresis on a 3.75% polyacrylamide slab gel containing 98% formamide, as described previously¹⁵. Microdensitometer scans of the autoradiogram of the wet gel are shown. Band 2 mRNA encodes the VSV G protein; band 3 encodes the N protein; and band 4 encodes the M and NS proteins¹⁶.

mRNA (band 3) and 30% of M and NS mRNAs (band 4).

To quantify the removal of 7-methylguanosine from the mRNAs, samples were digested with penicillium nuclease (PI), an enzyme which degrades RNA to 5' mononucleotides regardless of base or ribose modifications¹⁹. The 5'-terminal structures expected from PI digestion of untreated and β -eliminated VSV mRNAs are $\text{m}^7\text{G}^5'\text{ppp}^5'(\text{m})\text{Am}$ and $\text{ppp}(\text{m})\text{Am}$, respectively⁵. To separate these termini from the 5' mononucleotides, we subjected the digests to ionophoresis on DEAE paper at pH 3.5 (Fig. 2). Periodate oxidation and β -elimination resulted in the disappearance of radioactivity in $\text{m}^7\text{G}^5'\text{ppp}^5'(\text{m})\text{Am}$ and its appearance in $\text{ppp}(\text{m})\text{Am}$ (lanes 1 and 2); the latter migrates much more

slowly than the parent structure because of the loss of the positive charge on 7-methylguanosine and the gain of an additional negative charge from the unesterified phosphate. The structures of these nucleotides were demonstrated by their respective resistance and sensitivity to digestion with alkaline phosphatase digestion and by the identification of the products derived by venom phosphodiesterase digestion of each, as was done previously: $\text{m}^7\text{G}^5'\text{ppp}^5'(\text{m})\text{Am}$ $\xrightarrow{\text{venom}}$ pm^7G , Pi, $\text{p}(\text{m})\text{Am}$; $\text{ppp}(\text{m})\text{Am}$ $\xrightarrow{\text{venom}}$ PPI , $\text{p}(\text{m})\text{Am}$ (ref. 5). The low levels of pppA and pppG in the mRNA preparations are derived from a fraction of the non-polysomal VSV RNAs which terminate in 5' triphosphates⁵.

No residual 5'-termini containing 7-methylguanosine were detected from β -eliminated mRNA (Table 1) since all residual radioactivity migrating at the position of $\text{m}^7\text{G}^5'\text{ppp}^5'(\text{m})\text{Am}$ was sensitive to alkaline phosphatase. If 1% of the RNAs had contained the 5'-terminus $\text{m}^7\text{G}^5'\text{ppp}^5'(\text{m})\text{Am}$, we would have detected it. Figure 2, lane 3, and Table 1 show that incubation of the β -eliminated RNA in the reticulocyte cell-free system in the conditions of protein synthesis does not result in the addition of 7-methylguanosine to the 5' end; the end remains $\text{ppp}(\text{m})\text{Am}$.

Translation without 7-methylguanosine

The products of translation of these mRNAs in the reticulocyte cell-free system were resolved by polyacrylamide

Fig. 2 DEAE paper iontophoresis at pH 3.5 of nuclease P1 digests of VSV mRNA. Samples containing 10 μg of RNA were incubated with 0.01 μg of P1 nuclease (Yamasa Shoyu Co., Tokyo) in 5 μl of 0.01 M MES (2-N morpholino-ethanesulphonic acid, pH 6.2) for 30 min at 37°C . The limit digests of VSV mRNA were spotted on a sheet of Whatman DEAE (DE-81, 30×60 cm) paper and subjected to electrophoresis at 30 V cm^{-1} for 4 h in 5% acetic acid, 0.5% pyridine. Lane 1, untreated mRNA; lane 2, periodate-oxidised, β -eliminated mRNA; lane 3, periodate-oxidised, β -eliminated VSV mRNA purified after incubation in the reticulocyte cell-free system as described in Fig. 3.

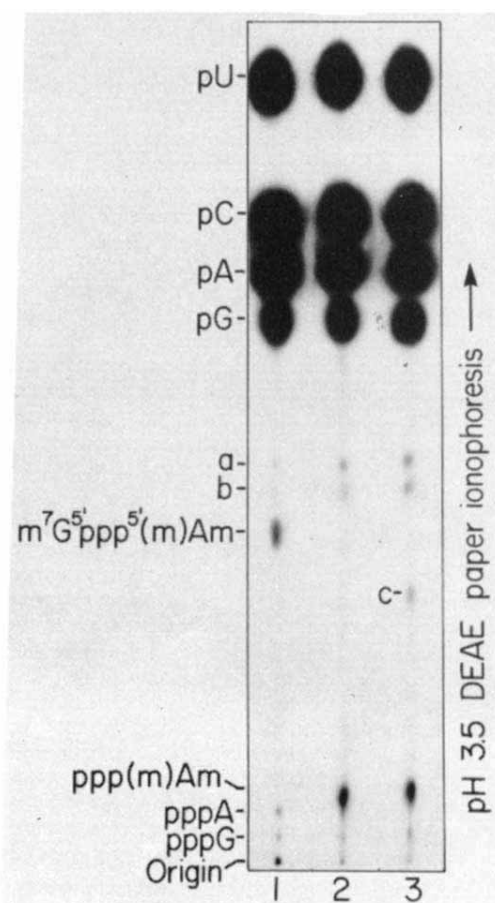


Table 1 Quantification of 5' termini

	HO-m ⁷ G ⁵ /ppp ⁵ -(m)Am-OH*	% Total c.p.m. in each structure ppp ⁵ -(m)Am-OH	ppp ⁵ Am-OH	pppG ⁵ OH	Total radioactivity (c.p.m.)
Untreated	0.17 (2141)	0.0006 (8)	0.019 (246)	0.018 (227)	1.26 × 10 ⁶
Periodate-oxidised, β-eliminated	<0.0017 (<20)	0.17 (1874)	0.021 (241)	0.019 (208)	1.11 × 10 ⁶
β-Eliminated (analysis after incubation in a protein synthesis reaction)	<0.0017 (<20)	0.16 (1788)	0.018 (212)	0.019 (210)	1.13 × 10 ⁶

The 5'-terminal structures and 5' monophosphates, separated as shown in Fig. 1, were cut out of the DEAE paper and radioactivity was determined in a scintillation spectrometer. For each structure c.p.m. are given in parentheses. Radioactivity in the spots labelled *a* and *b* (Fig. 2, lanes 1 and 2) was converted to P_i after alkaline phosphatase treatment and thus is either derived from modified nucleotides at internal positions in VSV mRNA or generated as an artefact of nuclease P1 digestion. The spot labelled (*c*) (Fig. 2, lane 3) appeared only in nuclease P1 digests of VSV mRNA which had been incubated in the reticulocyte cell-free system. Since half of the radioactivity in this compound was released as P_i after alkaline phosphatase digestion, it is presumably a P_i-resistant modified dinucleotide of the form pNpN.

* We corrected for background due to trailing of mononucleotides into the region containing m⁷G⁵/ppp⁵-(m)Am as follows. Radioactivity in this region was eluted with 30% triethylammonium bicarbonate (pH 7.8), lyophilised, treated with alkaline phosphatase, and subjected to electrophoresis at pH 3.5 on Whatman No. 3MM paper, as described previously⁵. The radioactivity in ³²P_i relative to that in the phosphatase-insensitive structure m⁷G⁵/ppp⁵-(m)Am (16%) was taken as the fraction of contamination with mononucleotides, and the numbers in the table have been corrected for this contamination. All radioactivity in the region of m⁷G⁵/ppp⁵-(m)Am from β-eliminated RNA (285 and 260 c.p.m.) migrated as ³²P_i after phosphatase treatment, and the numbers given in the table have been corrected to the limits of detection of remaining m⁷G⁵/ppp⁵-(m)Am.

gel electrophoresis. Figure 3*a* and *b* shows that synthesis of M, NS and N proteins is proportional to the concentration of added control RNA. Synthesis of the VSV G protein is presumably obscured by the large background peak of reticulocyte protein at 2 cm (compare Fig. 3*e*). Both periodate-treated and periodate-treated, β-eliminated mRNA direct the synthesis of authentic VSV M, NS and N proteins (Fig. 3*c* and *d*). Quantitation of these microdensitometer scans showed that periodate-treated RNA and periodate-treated, β-eliminated RNA directed the synthesis of 33% and 25%, respectively, of the amount of each of these proteins compared with the same amount of control RNA. The amount of protein synthesis directed by periodate-treated and periodate-treated, β-eliminated RNA was also directly proportional to added RNA concentrations (data not shown).

Because we were concerned that the reduced translation of the chemically treated VSV mRNA might in part be due to effects generated not by removal of the 7-methylguanosine but by internal oxidations of bases in the RNA as well as degradation, we performed control experiments on f2 phage RNA which had been mixed with the ³²P-labelled VSV mRNAs during the periodate-oxidation and β-elimination procedures. When translated in an *E. coli* cell-free system, periodate-oxidised, β-eliminated f2 RNA synthesised 80% of the amount of f2 coat protein (molecular weight 14,000) and only 50% the amount of RNA synthetase (molecular weight 50,000) as did untreated f2 RNA (data not shown). These experiments, as well as those showing degradation of VSV mRNAs described above, indicate that the level of translation in reticulocyte extracts after the periodate-aniline treatment must be regarded as a minimal estimate of the ability of VSV mRNA lacking 7-methylguanosine to function in protein synthesis.

Ribosome attachment

Two additional studies confirmed that VSV mRNA lacking 7-methylguanosine can participate in protein synthesis. After incubation in a reticulocyte extract in conditions of protein synthesis, 51% of control RNA is incorporated into polyribosomes; these contain, on average, 3.4 ribosomes per mRNA (Fig. 4*a*, Table 2). The same amount of periodate-treated, and slightly less periodate-treated, β-eliminated RNA is incorporated into polysomes. The decrease in the fraction of β-eliminated mRNA bound is due presumably to partial degradation of the RNA (Fig. 1). The average size of polysomes formed is reduced less than twofold compared with those from control RNA (Fig. 4*b* and *c*, Table 2). Assuming that the rate of polypeptide chain elongation and termination is the same for control and treated RNA, and that the average size of the VSV proteins

produced is the same (compare Fig. 3), this experiment suggests that the rate of ribosome attachment to mRNAs lacking 7-methylguanosine is reduced less than twofold.

This conclusion is substantiated by the experiments in Figs 5 and 6 which measure directly the rate and extent of incorporation of mRNA into 80S initiation complexes. Incubation of control VSV mRNAs with reticulocyte extracts in the presence of anisomycin (1 mg ml⁻¹), a specific inhibitor of polypeptide chain elongation²⁵ results in incorporation of 54% of the RNA into an 80S complex (Fig. 5*a*). This complex remains stable in a solution of 0.5 M NaCl and 0.03 M magnesium acetate, but no initiation complexes are produced if the incubation mixture is of this ionic composition (data not shown). Hence, the rate of formation of an 80S complex can be determined accurately by diluting the incubation mixture into a solution of 0.5 M NaCl and 0.03 M magnesium acetate, followed by zone centrifugation to isolate the 80S complex. Figure 5 shows that 53% of periodate-treated RNA and 37% of periodate-treated, β-eliminated RNA is incorporated into a salt-stable initiation complex. Again, the reduction in the extent of binding is due, presumably, to degradation of RNA. The time required for half-maximum binding of control RNA to ribosomes is 12 s, while that for either periodate-treated RNA or periodate-treated β-eliminated RNA is about 35 s (Fig. 6). A threefold reduction in the rate of binding to ribosomes was also observed when untreated and periodate-treated, β-eliminated forms of a single VSV mRNA, that encoding N protein, were compared (control RNA, *t*_{1/2} = 10.1 s; periodate-treated, β-eliminated RNA, 35.8 s; data not shown). This experiment rules out the possibility that the apparent reduction is due to preferential destruction of the larger VSV mRNAs by the chemical treatment.

The experiments reported here demonstrate that animal cell ribosomes can recognise the correct translation initiation sites on animal virus mRNAs from which the 5'-terminal 7-methylguanosine has been removed since such mRNAs direct the synthesis of authentic viral proteins. The changes which we observe in the ribosome binding of periodate-treated, and periodate-treated, β-eliminated mRNA are a threefold reduction in the rate of binding to ribosomes, a twofold reduction in average polysome size, and a 20–30% reduction in the total amount bound. The effect of the chemical treatments on the total amount of mRNA binding may be due only to mRNA degradation. Furthermore, we cannot rule out the possibility that the effect on the rate of binding may be due not to the removal of 7-methylguanosine but to nonspecific modifications introduced during periodate oxidation. Indeed, we have observed here that periodate oxidation on its own, which presumably leaves a dialdehyde

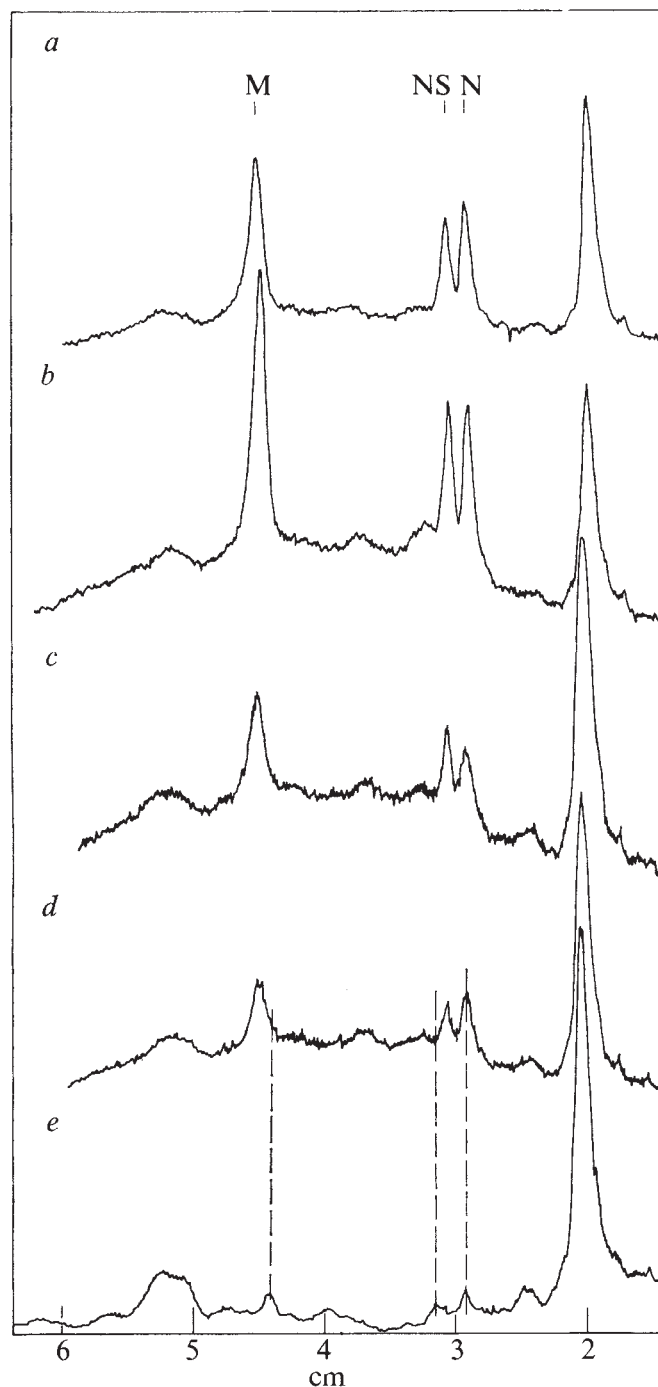


Fig. 3 Translation of VSV mRNAs. Conditions for protein synthesis in cell-free extracts of rabbit reticulocytes have been described in detail²³. Reactions (39 μ l) containing 7 μ Ci ³⁵S-methionine (200,000 mCi mmol⁻¹, New England Nuclear), 10⁻⁴ M each of 19 non-radioactive amino acids, and the indicated amounts of ³²P-labelled VSV mRNA. S-Ado-Hcy (S-adenosyl-homocysteine) was added to all reactions at a final concentration of 7.4×10^{-4} M to inhibit methylation. Incubation was at 30 °C for 60 min. Pancreatic RNase (5 μ g) was added, and incubation was continued for 5 min more. A sample (3 μ l) of the reaction was analysed on a 13% polyacrylamide slab gel as detailed by Laemmli²⁴. Shown are scans of autoradiographs (10-d exposure) of the fixed, dried gel. The region of the gel containing the globin 7–9 cm) is not shown. *a*, 0.3 μ g of control VSV mRNA (7.7 μ g ml⁻¹); *b*, 0.6 μ g of control VSV mRNA (15.4 μ g ml⁻¹); *c*, 0.6 μ g of periodate-treated VSV mRNA (15.4 μ g ml⁻¹); *d*, 0.6 μ g of periodate-treated and β -eliminated VSV mRNA (15.4 μ g ml⁻¹); *e*, no added RNA.

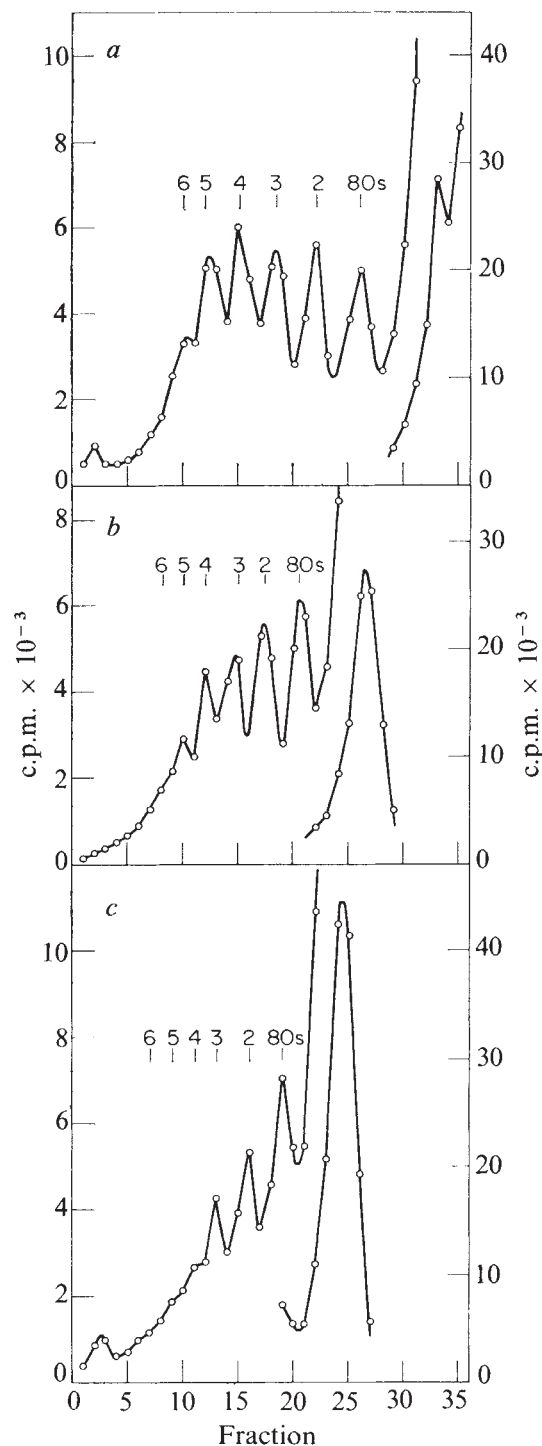


Fig. 4 Incorporation of labelled VSV mRNA into polysomes. Reticulocyte cell-free reactions (120 μ l) contained 10⁻⁴ M each of 20 amino acids, 7×10^{-4} M S-Ado-Hcy, and 1.5×10^5 c.p.m. (0.1 μ g) of ³²P-labelled control VSV mRNA (*a*); periodate-treated RNA (*b*); or periodate-treated, β -eliminated RNA (*c*). Incubation was at 30 °C for 5 min. Reaction tubes were chilled on ice, and 1.2 ml of HSB (0.5 M NaCl, 0.03 M Mg (acetate)₂, 0.02 M HEPES, pH 7.5) was added. The samples were layered on a 36-ml 15–30% (w/v) linear sucrose gradient, made up in HSB, and centrifuged in a Beckman SW27 rotor at 4 °C at 26,500 r.p.m. for 4 h. Samples were collected through a flow cell in a Gilford recording spectrophotometer directly into scintillation vials. The arrows represent optical density peaks of the reticulocyte polysomes which are due, predominantly, to the endogenous globin mRNA. Each sample was bleached with alkaline hydrogen peroxide and counted using Aquasol (New England Nuclear) in a Beckman scintillation spectrometer. In control reactions (which were not incubated at 30 °C) only 2.3% of the radioactivity was found in the monosome or polysome regions of the gradient (data not shown). Analyses of data from this experiment are in Table 2.

Table 2 Incorporation of ^{32}P -labelled VSV mRNA into polysomes

	Control RNA	Periodate-treated RNA	Periodate-treated, β -eliminated RNA
c.p.m. in monosomes or polysomes	77,910	57,612	46,346
$C = \sum_{i=1}^7 C_i$			
Proportionate number of ribosomes bound to VSV mRNA	260,720	162,913	105,769
$R = \sum_{i=1}^7 i C_i$			
Ribosomes per bound mRNA $r = C/R$	3.35	2.83	2.28
Total c.p.m. recovered from gradient C_t	152,764	115,220	118,840
Fraction of mRNA bound to ribosomes $C_B = C/C_t$	0.51	0.50	0.39

In calculating the data given in line 2, the amount of radioactivity in any region of a polysome gradient was multiplied by the number of ribosomes per polysome (i), that is, by the number of ribosomes translating each mRNA in that peak. Hence, the total number of ribosomes translating the mRNAs is proportional to the sum of this product over all regions of the polysome gradient (line 2). Since the total radioactivity in polysomes (line 1) is proportional to the number of VSV mRNAs being translated, the average number of ribosomes per bound mRNA is given by line 3.

on the 7-methylguanosine as well as on the 3'-terminal adenosine of the mRNA, has virtually the same effect on reducing the rate of mRNA binding to ribosomes as does periodate-oxidation followed by β -elimination, which removes the oxidised nucleosides from the mRNA.

Conflicting data

Our conclusions conflict with those drawn in previous studies on translation of methylated and unmethylated VSV mRNAs synthesised *in vitro* by the virion-bound VSV polymerase. It was reported that VSV mRNAs synthesised *in vitro* terminate in $\text{G}^{\gamma}\text{ppp}^{\gamma}\text{Ap}^{20}$ and that translation of this RNA in the wheat embryo system will not occur unless methylation at the N^7 -position of the guanosine has occurred before translation¹¹.

Attempting to reconcile these results with our own, we synthesised VSV RNA *in vitro* in the presence of S-adenosyl homocysteine and verified that the product RNA contains the 5'-terminus $\text{G}^{\gamma}\text{ppp}^{\gamma}\text{Ap}$. We found, however, that this product RNA does not contain any discrete VSV mRNA species comigrating with authentic VSV mRNA on formamide polyacrylamide gels. Instead, 80% of the product RNA is larger than the N protein mRNA and heterogeneous in molecular weight, while the remaining 20% is present as bands running just faster than (approximately 100–200 nucleotides shorter than) the authentic VSV mRNAs (unpublished observations of M. Brock and J. Rose). In contrast, 80–90% of the *in vitro* product synthesised in the presence of S-adenosyl methiodine does comigrate with authentic VSV mRNAs. These results suggest that methylation is involved in synthesis or processing of VSV mRNAs into the correct poly(A)-containing mRNA species. The ability of these RNAs to function in *in vitro* protein synthesis is under study.

Extensive experiments carried out with reovirus mRNAs have also indicated a requirement for a 5'-terminal 7-methylguanosine in translation and ribosome binding¹². If the methylated and unmethylated mRNAs compared in these experiments differ in no way other than the presence

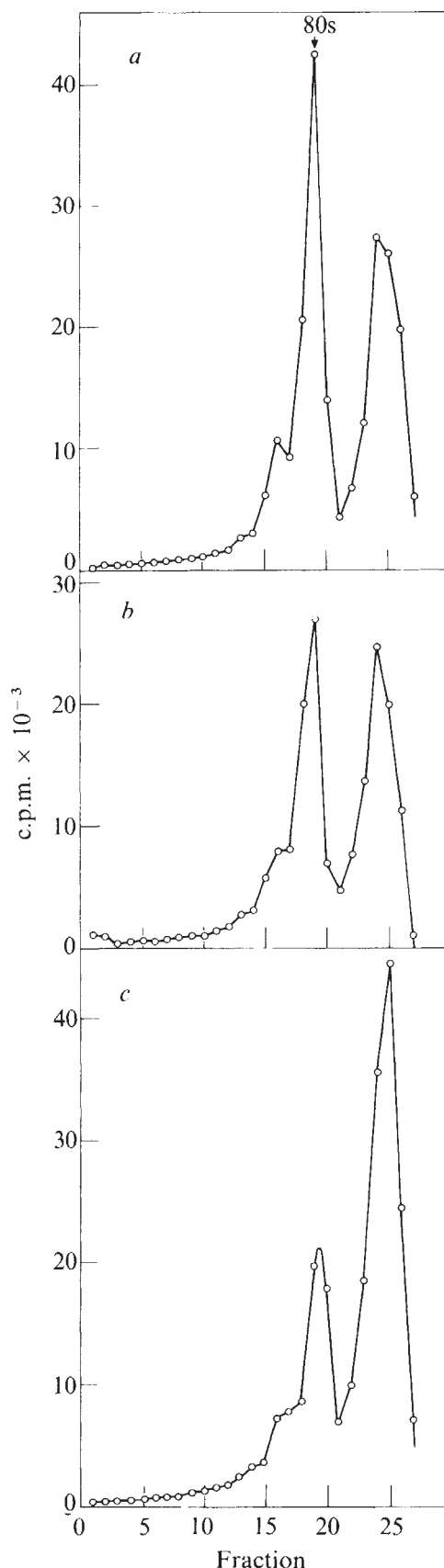


Fig. 5 Binding of ^{32}P -labelled VSV mRNAs to reticulocyte ribosomes. Reactions contained anisomycin (1 mg ml^{-1}), a specific inhibitor of polypeptide chain elongation²⁵ but were otherwise identical to those of Fig. 4. Gradient centrifugation and counting of the samples was as in Fig. 4. In reactions not incubated at 30°C , 2.1–2.6% of the ^{32}P -labelled RNA was found to sediment faster than 40S (data not shown). The percentage of added ^{32}P -labelled mRNA bound to ribosomes after incubation was 54% (a); 53% (b); 37% (c). The concentration of VSV mRNA in each reaction was $1 \mu\text{g ml}^{-1}$.

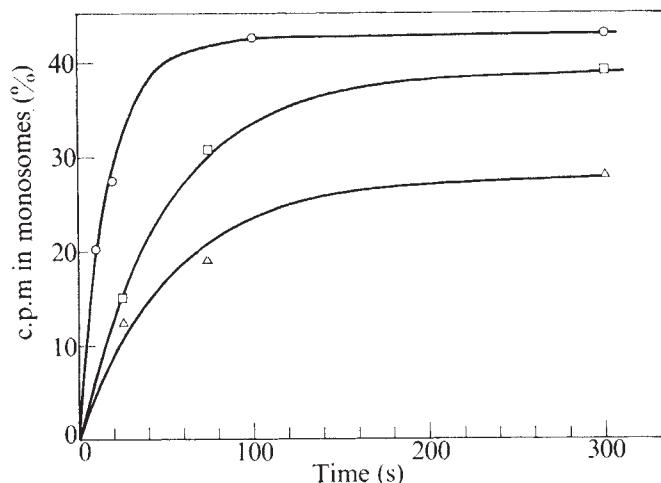


Fig. 6 Rate of binding of VSV mRNAs to reticulocyte ribosomes. Reactions (330 μ l containing about 10^6 c.p.m. of 32 P-labelled control VSV mRNA ($\circ$), periodate-treated RNA ($\square$), or periodate-treated and β -eliminated RNA ($\triangle$) were identical to those used in the study of Fig. 5. Incubation was at 30 $^{\circ}$ C. At times indicated, 75 μ l samples were added to 0.5 M HSB at 0 $^{\circ}$ C. Samples were layered on 13-ml linear 15–30% sucrose gradients in HSB and centrifuged for 3.4 h at 40,000 r.p.m. in the SW40 rotor at 4 $^{\circ}$ C. Collection and counting was as in Fig. 5. The percentage of the radioactive RNA found in the 80S region of the gradient is plotted. A background of 2.1%, observed with unincubated samples, has been subtracted from each point. The solid curves are those of the first-order binding equation

$$C = C_{\infty} \left[1 - \left(\exp \frac{-t \ln 2}{t_{1/2}} \right) \right]$$

where C is the percentage of RNA bound to monosomes at time t , C_{∞} is the maximum value of C , and $t_{1/2}$ is the time required for half-maximum binding of the RNA. For each RNA sample, C_{∞} and $t_{1/2}$ were calculated by a least-squares program using the observed data points. These values are: control RNA, $C_{\infty} = 42\%$, $t_{1/2} = 11.7$ s; periodate-treated RNA, $C_{\infty} = 39\%$, $t_{1/2} = 34.6$ s; periodate-treated and β -eliminated RNA, $C_{\infty} = 28\%$, $t_{1/2} = 36.7$ s.

or absence of a 5'-terminal 7-methylguanosine as reported¹², then the results imply a more intimate involvement of 7-methylguanosine in translation of reovirus mRNAs than we observe for VSV mRNAs. This perhaps indicates a close proximity of the reovirus 5' terminus to the translation initiation site, a situation already documented for the coat protein mRNA of brome grass mosaic virus²¹.

We initially had great difficulty recovering translatable VSV mRNA after periodate-oxidation and β -elimination, and clearly did not completely overcome the problem of

degradation. Thus, we suggest that similar studies indicating a requirement for 7-methylguanosine in translation of cellular mRNAs^{13,22} should be more carefully controlled for possible nonspecific effects of the chemical procedure.

The model we favour is that in which the terminal 7-methylguanosine has different effects on translation of different mRNAs. For some mRNAs, for example, poliovirus RNA and VSV mRNAs, it is clearly not essential, but it may facilitate ribosome attachment to VSV mRNA. For other mRNAs, a 5'-terminal 7-methylguanosine may be essential. It is also possible that wheat embryo ribosomes—used for much of the previous work—differ from animal ribosomes in their dependence on the 7-methylguanosine residue. Indeed, we have observed as much as a tenfold reduction in translation of periodate-treated, β -eliminated VSV mRNA in the wheat embryo system, perhaps indicating a more important role for 7-methylguanosine in this heterologous system. Thus, both the mRNA in question and the source of the translational components may be important factors in determining the effect of the 5'-terminal 7-methylguanosine.

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letters to nature

X-ray pulses from globular clusters

GIANT X-ray pulses have been observed recently by several groups^{1–7} from sources located in a number of celestial positions. Two of these sources may plausibly be identified with the globular cluster X-ray sources: NGC6624 and NGC1851. In several cases^{2,7} the pulses seem to occur in approximately periodic sequences; in other sources the intervals are irregular,

but in one case there is a relation between pulse strength and interpulse interval. The non-burst behaviour of globular cluster X-ray sources has been interpreted^{8,9} in terms of massive ($\gtrsim 10^3 M_{\odot}$) black holes powered by accretion from a disk supplied by cluster gas. We suggest here that compact stars bound to the central massive black hole and orbiting through the accretion disk may produce, by several possible mechanisms, giant pulses. These pulses might be expected to exhibit a variety

of types of X-ray behaviour corresponding to the uncertain characteristics (for example, density and spatial extent) of the individual accretion disks at the different points at which the orbits of the bound compact stars intersect the disks. We note that there may be more than one class of sources of giant X-ray bursts and that our speculation refers only to those sources in globular clusters.

We can easily estimate the expected orbital period of the star closest to the central black hole. The rate at which stars are captured has been computed by several authors¹⁰⁻¹². It is to an order of magnitude, and neglecting dimensionless constants,

$$\dot{N} \lesssim \left(\frac{v_{r.m.s.}}{R} \right)_{\text{core}} \left(\frac{M_{\text{BH}}}{M_{\text{core}}} \right)^3 \simeq 10^{-7.0} (M_{\text{BH}}/10^3 M_{\odot})^3 \text{ yr}^{-1} \quad (1)$$

where 'core' refers to characteristics of the globular cluster core. (The rate at which the tightly-bound orbits relevant to the present discussion are populated may be less than this, but our results are, fortunately, insensitive to N .) At small distances from the hole, gravitational radiation dominates the two-particle scattering and the drag caused by interaction with the disk. Stars with period, P , will spiral quickly towards the hole on a time scale

$$\tau_{\text{rad}} = \left(\frac{R}{R_{\text{GR}}} \right) = 10^{5.7} (M_{\text{BH}}/10^3 M_{\odot})^{-2/3} \times \\ \times (M_{\star}/M_{\odot})^{-1} \left(\frac{P}{1 \text{ h}} \right)^{8/3} \text{ yr} \quad (2)$$

The time that a star spends at any given radius decreases rapidly with decreasing radius ($P \propto R^{3/2}$; $\tau_{\text{rad}} \propto R^4$). Hence we find, by multiplying together equations (1) and (2), the period of the innermost stellar orbit that most likely contains one star

$$P \gtrsim 4 (M_{\text{BH}}/10^3)^{-7/8} \text{ h} \quad (3)$$

Thus a clock with a period of hours to one day is natural in this model, in agreement with observations^{2,7}. (For main-sequence stars, tidal effects set a lower limit of this order to the orbital period.) Of course, the manifestation of this period requires various things about the accretion disk.

The duty cycle for 3U1820-30 is apparently very small ($\sim 10^{-3}$) so the interaction between the orbiting star and the disk would have to be relatively brief for this source, that is, a very thin disk ($\lesssim 10^{8.5}$ cm) would be required. At least two mechanisms for producing the pulses come to mind: (1) a small, hot, shocked area will be produced twice each orbit as the innermost star crashes through the disk. This might be seen directly: its properties will depend on whether it is caused by an ordinary star losing mass by tidal stripping and producing a bow shock (P. Silk, personal communication) or, more likely, a compact star producing an observable accretion wake; (2) ripples will proceed from the shocked zone towards the centre of the disk producing, when the wave train reaches the innermost stable orbit, extra accretion and luminosity. Depending on the orientation of the system with respect to our line of sight, and on whether or not the disk is optically thick to X rays, one would expect to see one or two pulses per orbital period. Irregularities and instabilities in the disk could produce the observed² r.m.s. phase jitter of $\lesssim 0.04$ per period.

There are certain additional observational consequences of this model: (1) globular clusters in our Galaxy can be far away (~ 10 kpc) so that one would not be surprised if some of the sources near the galactic centre or at low galactic latitude were associated with optically obscured globular clusters; (2) the galactic latitude dependence of the sources we consider should

be similar to that of the known globular clusters except there may be a number of optically undiscovered X-ray globular clusters in the direction of the galactic centre (scale size ~ 1 kpc by analogy with globular clusters in Andromeda); (3) more than one period per source might be found if there were more than one star orbiting near the hole. If this were discovered it would be difficult to explain on the usual binary hypotheses; (4) as noted previously⁸, no eclipses should be seen according to this model and (5) a small odd-even effect caused by non-zero orbital eccentricity might be observable.

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Frequency variation of equatorial ionospheric scintillations

THE fluctuations in the signal strength of v.h.f. and u.h.f. transmissions from satellites received at ground level contain valuable information about ionospheric irregularities. These fluctuations, usually called scintillations, depend on the frequency of transmission, characteristics of the irregularities and the angle of incidence. At equatorial latitudes, there have as yet been few such observations, and there are reasons to expect the scintillation characteristics at equatorial latitudes to be quite different from those at higher latitudes because the ionospheric irregularities (mainly spread F irregularities) responsible for scintillations differ widely in their characteristics with latitude. It is also well recognised that the equatorial region is a strong scintillation zone. At Trivandrum (dip $0^{\circ}57'S$, geographic longitude $76^{\circ}57'E$), a station close to the magnetic equator, amplitude and phase scintillations of the radio beacon transmissions on 40, 140 and 360 MHz as well as the amplitude scintillations of the 860-MHz television downlink from the geostationary satellite ATS-6 have been recorded since August 1975. Here we present preliminary results on the frequency variation of the scintillation index.

In Fig. 1 a typical record of scintillations on all the four frequencies is shown. The records at 40 and 140 MHz are synchronous whereas those on 360 and 860 MHz are not. It is a general observation that scintillations are faster at lower frequencies. The quasi-periods of fluctuations are in the range of few seconds to few tens of seconds, the quasi-periods being shorter at lower frequencies. Also, scintillations occur more frequently and earlier in the night at lower frequencies. We here consider only nocturnal scintillations which can be attributed to the phenomenon of spread F. For this study, data recorded during six nights in the month of October 1975 have been used. Of these, scintillations were observed on four nights at all four frequencies and on the other two nights scintillations were observed on 40, 140 and 360 MHz only.

Using the amplitude scintillation data on all the frequencies, the S_4 scintillation indices¹ have been evaluated. For this, the length of the data sample considered corresponds to ~ 10 min, which is sufficiently long not to cause any error in the S_4 indices, considering the quasi-periods of the fluctuations.

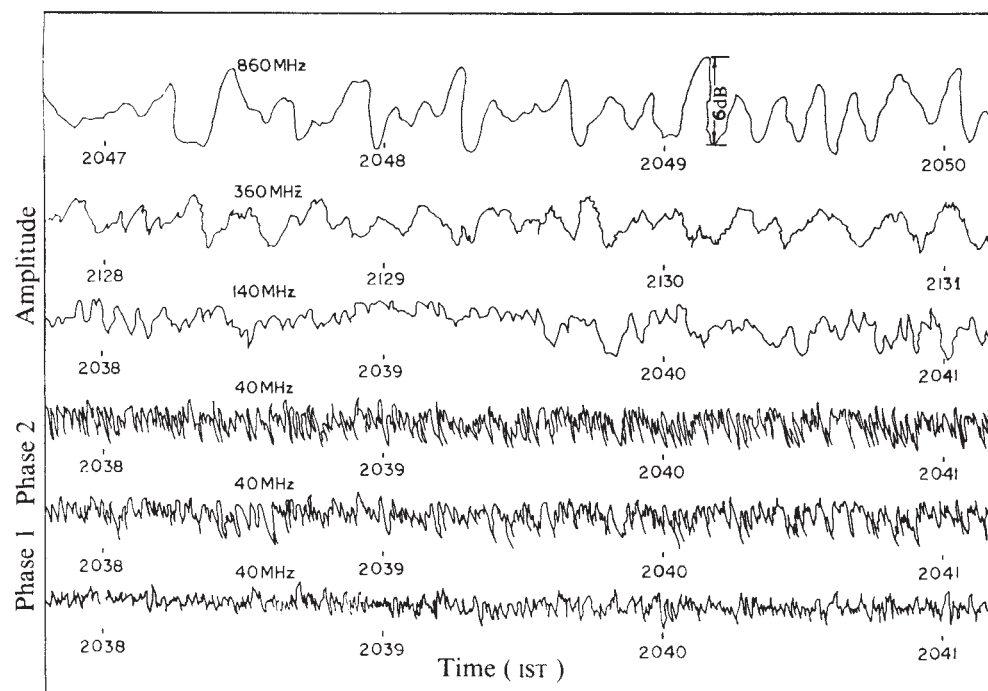


Fig. 1 Scintillations of ATS-6 transmissions recorded at Trivandrum. All traces taken on October 21, 1975 except the second (360 MHz), which was taken on October 24, 1975.

It is reasonable to assume that this interval is short enough for the scintillation characteristics not to vary within this interval except perhaps during onset and decay of scintillations. It may be noted that the onset and decay periods are not included in the present analysis.

The frequency variation of S_4 can be represented by

$$S_4 = \frac{K}{f^n}$$

where f is the exploration frequency, n is the frequency index and K is a constant depending on the scintillation conditions only. This equation can be rewritten as

$$\log_{10} \left(\frac{S_4)_1}{(S_4)_2} \right) = n \log_{10} \frac{f_2}{f_1} \quad (1)$$

where $(S_4)_1$ and $(S_4)_2$ are the scintillation indices corresponding to the frequencies f_1 and f_2 . The quantities $\log_{10}[(S_4)_1/(S_4)_2]$ and

$\log_{10}[(f_2)/(f_1)]$ are plotted in Fig. 2. It has been found that the points (circles and crosses in Fig. 2) fall around two straight lines having different slopes. The slope of line A is 1.2 and that of line B is 0.46. The line A corresponds to frequencies > 140 MHz whereas the line B includes data points at 40 but not 860 MHz. A careful examination of the data showed that the S_4 indices can be divided into two groups depending upon whether the S_4 index at 360 MHz is greater or less than ~ 0.5 . The conditions corresponding to $S_4|_{360} > 0.5$ can be termed as strong scintillation conditions and those corresponding to $S_4|_{360} < 0.5$ as relatively weak scintillation conditions. The lines A and B (Fig. 2) represent weak and strong scintillation conditions respectively. It is also found that when the $S_4|_{140}$ is ~ 0.8 , $S_4|_{40}$ is also about the same (the triangle point in Fig. 2) indicating no frequency variation of the S_4 index in the frequency range 40–140 MHz.

Recently Rufenach² has shown theoretically that, for the weak scattering case, n is between 1.4 and 1.6 depending on the exponent of the irregularity spectrum in the range 3.5–4.5. The multiple scattering case has been considered on theoretical grounds by Yeh *et al.*³ They had shown that n is $\ll 1$ for lower frequencies and it approaches a value of 1.5 at higher frequencies. The results presented here seem to agree with these theoretical considerations. The transition from the low to high frequency index appears to occur in the frequency range 140–360 MHz. The reason for the low value of n obtained here for the weak scattering case as compared to that given by Rufenach² and Yeh *et al.*³ is perhaps that line A (Fig. 2) representing the weak scatter case is somewhat vitiated by $S_4|_{140} > 0.5$. A more detailed discussion on the theoretical interpretation of these results will be presented elsewhere.

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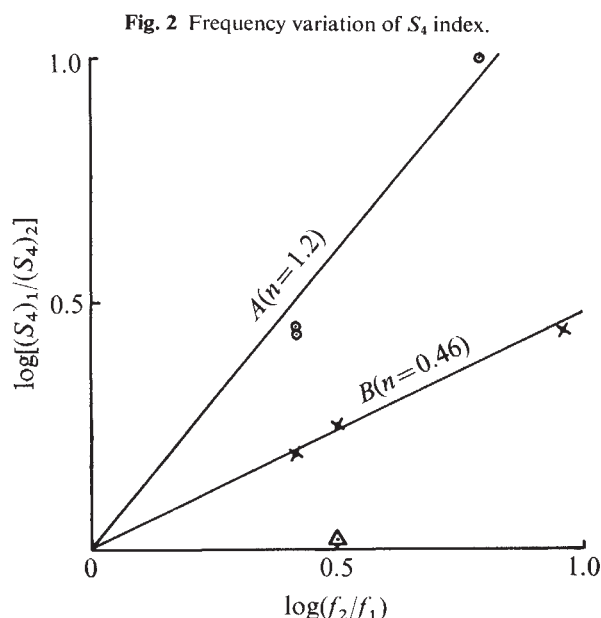
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Ocean temperatures and large scale atmospheric variations

WE here analyse some observations of sea surface temperatures to investigate several aspects of large scale ocean-atmosphere interactions, in particular El Nino and the Southern Oscillation.

Monthly mean sea surface temperature observations for most of the Pacific Ocean between 50°N and 20°S for the period 1949–73 were given an empirical orthogonal function analysis based on values at 160 grid points, each centred in an area 10° longitude by 5° latitude¹. This analysis produces orthogonal spatial functions and corresponding orthogonal time coefficients which are the best linear representations (predictors) of the data². The functions and coefficients may be thought of as analogues of the more familiar Fourier components and amplitudes, except that the empirical functions are determined from the interrelationships within the data itself.

Figure 1 illustrates the most important empirical function of the deviations of the sea surface temperatures from the 25-yr monthly means. This function accounts for 23.1% of the total variance in the field. The temperature deviation explained (predicted) by this function for a given place and time is simply the product of the value of the function at that place and the value of the time coefficient for the month in question. The spatial pattern of this function reveals a large scale effect in which the equatorial and eastern Pacific vary in phase whereas the north-central and western Pacific are out of phase. The time series is a good measure of the occurrence and strength of the so-called El Nino (and its inverse), which is the name given to periods of warmer(cooler)-than-average water off Peru and along the equator.

The ultimate cause of the El Nino modulation is unknown, although it is believed to be closely linked to the equatorial wind-driven oceanic circulation. The pattern in Fig. 1 suggests an hypothesis for the termination of the equatorial warm-water periods. Higher equatorial sea-surface temperatures would tend to increase the rate of latent heat release in the tropical troposphere³, and this may be associated with greater poleward atmospheric fluxes of

heat and angular momentum⁴. These tendencies, coupled with an increased meridional subtropical atmospheric temperature gradient induced by the sea temperature anomalies, would result in strengthened mid-latitude westerlies and equatorial easterlies. The latter would act to increase cold upwelling along the equator and reduce the transport of warm water from the west towards Peru by the equatorial countercurrent⁵. The result would be the destruction of the El Nino conditions. A similar process was shown to be quite effective in increasing the easterlies in a numerical experiment by Rowntree⁶. This hypothesis is supported by the fact that, as indicated in Fig. 1, El Nino generally ends in the Northern Hemisphere winter when the meridional fluxes in that hemisphere are a maximum⁷. Such a negative feedback would imply that any causal mechanism proposed for El Nino must incorporate a persistence sufficient to explain the 6–18 month existence of the warm conditions.

El Nino is closely related to or even a part of another phenomenon called the Southern Oscillation. The latter is a negative correlation of atmospheric pressure fluctuations in the south-eastern Pacific with those in the Indonesian region. Lower pressures over the eastern Pacific tend to accompany warm water there and higher pressures over Indonesia. An empirical orthogonal analysis of tropical sea level pressure, temperatures, and rainfall by Kidson⁸ shows the largest fraction of the longer term variability in these fields occurs in Southern Oscillation modes. Variations of the time coefficients of these modes are quite similar to those of Fig. 1 for the period of Kidson's study, 1951–60. We have also compared the entire record of sea temperature coefficients with the variations of sea level pressure at Darwin, Australia, which has been used as a simple index of the Southern Oscillation⁹, for several lags and leads. The correlations at zero lag and when the sea temperatures lead the pressures by one month are both 0.55. The correlation when the pressure changes lead those of the sea temperatures by a month is 0.51, which is significantly different at the 95% confidence level.

Our sea temperature-time series also bears a remarkable resemblance to that of mean temperatures of the tropical troposphere recently reported by Angell and Korshover¹⁰ for 1958–73, as shown in Fig. 1. The only major discrepancy is for 1964–65 when the tropospheric temperatures remain below the mean whereas the sea temperatures are above. In

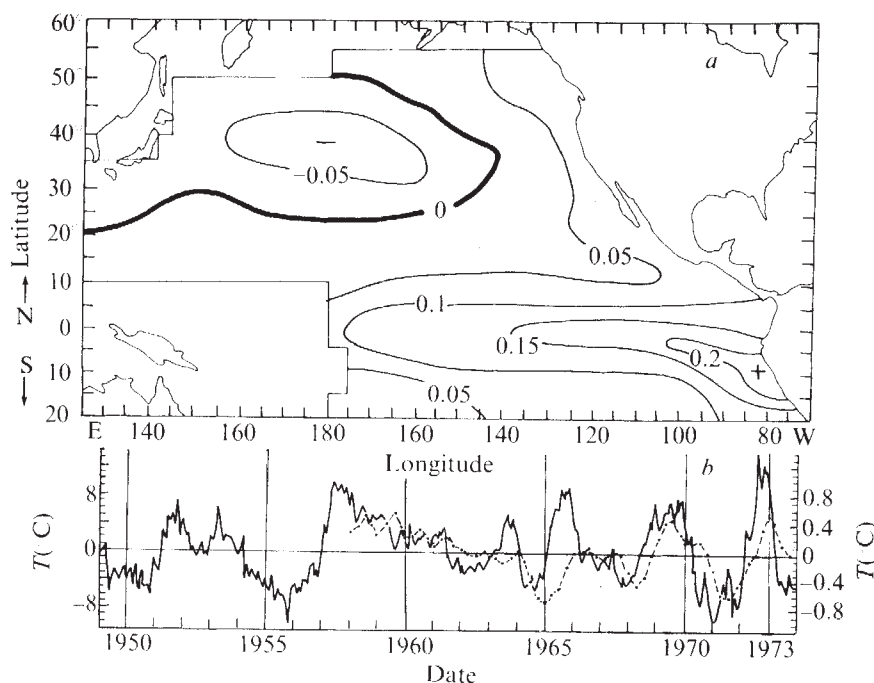


Fig. 1a, Empirical orthogonal function of sea-surface temperatures identifying the El Nino mode, and **b**, its corresponding time coefficients (solid line and left hand scale) and tropical troposphere mean temperatures (dotted line and right hand scale). The latter are derived from three-month means of 700–300 mbar thickness data for 14 stations situated near 20°N and 20°S, centred at January, April, July, and October¹⁰. Tics in the time series identify January of the respective year starting with January 1949 and ending with December 1973.

fact, the correlation coefficient between the two variables is 0.74 for the whole period when the sea temperatures lead the air temperatures by 6 months. This correlation increases to 0.80 when the years 1964–65 are excluded from the calculation. Although there are several possible reasons for the breakdown in correlation in 1964–65, we discuss here only two. One hypothesis is that sea temperatures remain low during this period in other oceans thus holding tropospheric temperatures down. Preliminary results of an analysis of sea temperatures in the Atlantic indicate, however, a small warming rather than cooling during 1965. A second hypothesis is that the difference is associated with a surface cooling resulting from the large increase in stratospheric aerosol which followed the eruption of Mt Agung in March 1963. The additional aerosol was associated with a temperature increase in the tropical stratosphere of 2–6 °C which took several years to disappear¹¹. Direct solar radiation measured at the ground was concomitantly diminished¹². Since the middle tropical troposphere is mainly heated through latent heat liberation which is decreased during periods of reduced solar surface heating, part of the tropospheric cooling in 1964–65 could probably be ascribed to the effects of Mt Agung. The magnitude of that effect remains an open question.

In addition to the six month phase lag which we have shown exists between tropical sea surface and tropospheric temperatures, Angell and Korshover report that tropospheric temperatures at high latitudes change six months later than those in the tropics. Although there are a number of possible factors that may introduce these lags, such as meridional oceanic transport processes and polar ice cap changes, separation of cause and effect is not possible at this time. Careful diagnostic studies and continuous monitoring of both ocean temperatures and volcanic eruptions, however, may enable us to use these phase lags to advantage in seasonal forecasting.

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Long term variations in western tropical Pacific cyclogenesis associated with the Southern Oscillation

THE 'Southern Oscillation', as statistically defined by Sir Gilbert Walker, is the barometrically recorded exchange of air mass in tropical latitudes around the complete circumference of the globe^{1,2}. Indices of Southern Oscillation

based on the surface pressure differences between the Indonesian equatorial low (measured at Darwin, Australia) and the South Pacific subtropical high (considered the Southern Oscillation's eastern core and measured at Easter Island) have been used by Quinn^{3–5}, who relates fluctuations in these indices to anomalous equatorial rainfall and occurrences of 'El Niño', (the accumulation of anomalously warm water in the eastern tropical Pacific). In this paper, we use 12-month running means of monthly mean surface pressures over three areas across the northern tropical Pacific as an index of the Southern Oscillation index, and we find that pressure convergences in this index are associated with years of relatively low numbers of western Pacific tropical cyclones.

Bjerknes² describes a major mechanism in the operation of the Southern Oscillation in the zonal atmospheric circulation which occurs over the equatorial Pacific between the ascending branches of the Hadley cells of the North-east and South-east Trade Winds. The normal sea surface temperature condition of warm water in the western tropical Pacific and cooler water (caused by equatorial upwelling and South American coastal upwelling) in the eastern tropical Pacific drives a zonal convective circulation along the equator between these two regions. The distinguishing feature of this particular east-west flow is the potential energy release involved in the dry descent of colder air over the eastern and central tropical Pacific combined with the ascent of warm moist air over the western tropical Pacific.

Anomalous warming of the eastern tropical Pacific sea surface has been related by Wyrtki⁶ to increased transport of warm western tropical Pacific water into the eastern tropical Pacific by means of the equatorial countercurrent. These eastern tropical Pacific warmings, in the extreme being referred to as 'El Niño', seem to occur aperiodically from 2 to 7 yr apart. The resultant decrease in equatorial sea surface temperature gradient across the Pacific reduces or halts the Walker Circulation, and is associated with the decrease of Southern Oscillation index, that is, a reduction of the difference between surface pressures at Darwin, Australia and Easter Island. What initiates a warming trend is not yet understood.

The situation of a well established Walker Circulation, with the ascent of warm moist air in the tropical western Pacific over a warm sea surface, enhances the first of the three conditions required for intense tropical cyclone formation. As described by Palmen and Newton⁷, the three conditions are: "(1) Sufficiently large sea areas with a water temperature so high that moist air lifted from the lowest layers of the atmosphere (with almost the temperature of the sea surface), expanding pseudo-adiabatically, remains considerably warmer than the surrounding undisturbed atmosphere at least up to a level of ~ 12 km. (2) A Coriolis parameter larger than a certain minimum value, thus excluding a belt of ~ 5° to 8° latitude on either side of the equator. (3) Weak vertical wind shear and correspondingly, weak baroclinity in the basic current in a deep tropospheric layer."

Ramage⁸ further notes that over the Pacific, near-equatorial troughs coincident with sea surface temperature maxima are the primary spawning grounds of almost all hurricanes. The preconditions of Palmen and Newton⁷ are then related to these troughs. Condition (1) is satisfied along the trough axis with the warmest sea surface temperature. Condition (2) is satisfied when the trough is > 5° from the equator where vortices can develop. Condition (3) is also satisfied, since weak vertical shear occurs above the sea surface temperature maximum associated with the trough axis.

It can be reasoned that a strong convective Walker Circulation, driven by the thermally derived pressure gradient between the warm western equatorial Pacific and

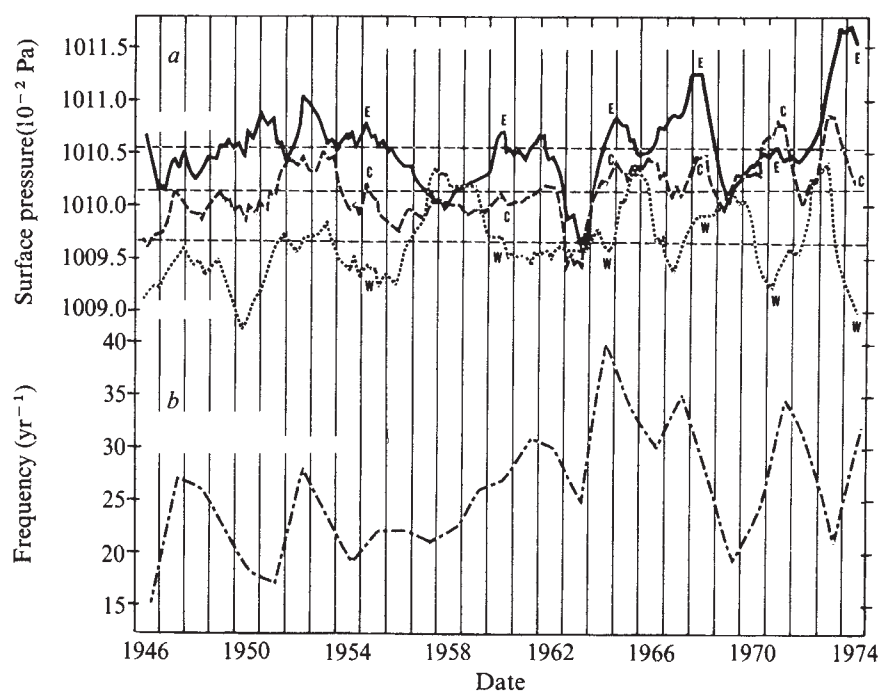


Fig. 1 Time series (1946-74) of 12-month running mean surface pressures (10^{-2} Pa, $1\text{ Pa} = 10^{-2}\text{ bar}$) for EASTROPAC (E), CENTROPAC (C), and WESTROPAC (W) and b, frequencies of western Pacific tropical cyclones.

a cooler eastern equatorial Pacific, might reinforce the conditions of tropical cyclone development in the near-equatorial trough in the western Pacific.

The question thus arises whether an eastern tropical Pacific sea surface temperature warming with the resultant decline in Walker Circulation and decreased ascent of warm moist air in the tropical western Pacific can be associated with a decline in the total number of tropical cyclones in the western Pacific for the given 'warming' year.

Twice-daily surface pressure analyses from 1946 to 1974 were used in the present study. The data were digitised in a 63×63 polar stereographic grid (grid size at 60°N is 381 km). The manually analysed charts were digitised at the National Climatic Center (1946-55, 1960-62) and in the National Center for Atmospheric Research (1956-59). From January 1963 onwards, the numerically analysed surface pressure charts from Fleet Numerical Weather Centre were used. The properties of these digitised surface pressure data are further described by Larson⁹.

Monthly means of surface pressure are computed for each grid point and each individual month, with emphasis mainly on three areas in the tropical Pacific in a latitudinal band, 0° to 15°N : The EASTROPAC area reaches from the Central American coast to 150°W ; the CENTROPAC area lies between 150°W and 165°E ; and the WESTROPAC area extends from 165°E to the Asian coast. The 0° to 15°N latitudinal band includes the main portion of the northeast tradewind zone, north equatorial current, and equatorial countercurrent. The monthly means as well as 12-monthly running means of surface pressure are computed for these three different areas; the 12-month running means are computed to eliminate most of the seasonal cycle and depict the long term ($> 1\text{ yr}$) changes.

Plotted together (Fig. 1a) the 12-month running mean surface pressures converge and diverge in good agreement with Quinn's³⁻⁵ Southern Oscillation index and Wyrski's⁶ equatorial countercurrent index (Harding and Larson¹⁰). This indicates that the data represent a manifestation of the Southern Oscillation, north of the equator.

The total numbers of tropical storms plus typhoons in the western Pacific for each year, 1946-74, according to Hamilton¹¹, are plotted to give a measure of interannual variations in tropical cyclogenesis.

More rigorous data treatment is not attempted because of the variety of available sources of pressure analyses and of the increased detectability of tropical cyclones with the advent of satellite meteorology in recent years.

Figures 1a and 1b present the 12-month running mean surface pressure plots for EASTROPAC, CENTROPAC and WESTROPAC as well as the yearly tropical cyclone numbers. The convergences of surface pressure between EASTROPAC and WESTROPAC (associated with EASTROPAC sea surface temperature warming (Wyrski⁶, Harding and Larson¹⁰)) since 1956 are associated with the minima in the number of tropical cyclones in the corresponding years. This is as expected, in as much as these pressure convergences indicate decreases or stoppages of Walker Circulation, and the resulting decrease in the amount of warm moist air ascending in the tropical western Pacific. Also to be noted are the tropical cyclone maxima occurring with the greatest EASTROPAC-WESTROPAC pressure differential, which indicate strong Walker Circulation and well maintained ascension of warm moist air in WESTROPAC.

The results of this study indicate that fluctuations in the Southern Oscillation seem related to interannual variations of tropical cyclogenesis in the western Pacific. The mechanism which connects these phenomena is probably the large scale release of potential energy caused by the thermally driven equatorial Walker Circulation. High pressure differential between the EASTROPAC and WESTROPAC represents good Walker Circulation and is associated with interannual maxima in western tropical Pacific cyclogenesis. Pressure convergence of EASTROPAC and WESTROPAC represents little or no Walker Circulation, and is associated with tropical cyclone minima.

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Three late-Devensian sites in West-central Scotland

PRELIMINARY investigations of late-Devensian sediments that include moss-sedge peat and moss detritus suitable for radiocarbon dating indicate movement of thick layers of till on the sides of drumlins in west-central Scotland since ~11,150 b.p. Two of the sites investigated are in inter-drumlin depressions on the north-eastern side of Glasgow: at Springburn (NS 609678) and Robroyston (NS 633676). The third site is between Paisley and Irvine on the floor of the Lochwinnoch Gap at North Kerse (NS 339556). The Quaternary sedimentary sequences at the three sites are shown in Fig. 1.

At Robroyston the silt underlying the till contains sparse fruit stones of a pondweed, *Potamogeton* cf. *pectinatus* L., a submerged aquatic plant of base-rich water, but virtually no pollen. Abundant remains of the algae *Botryococcus* and *Pediastrum* were found with a spore of the terrestrial, calcicolous moss genus *Encalypta* and numerous derived Carboniferous spores. The peat, composed of the moss *Scorpidium scorpioides* (Hedw.) Limpr. (another indicator of base-rich water) and a sedge (*Carex* sp.), has a pollen flora overwhelmingly of Cyperaceae but with 13 other taxa of herbs and dwarf shrubs (Table 1). The radiocarbon-dated material was the *Scorpidium-Carex* peat which, on careful examination, showed no Carboniferous contamination.

The lower peat at Springburn, consisting of pure *Scorpidium scorpioides*, has a pollen flora fundamentally similar to that of Robroyston in its large proportion of Cyperaceae, its range of herbs and its very low proportion of trees (Table 1). The upper peat contains mixed oak forest pollen with high *Alnus*, *Corylus* and ferns. The lower radiocarbon-dated sample was the *Scorpidium* peat (no

sign of Carboniferous contamination) and the upper was a highly decomposed peat.

The two sub-till peats, from Robroyston and Springburn, indistinguishable in age and very similar in botanical content, point to identical, if perhaps very local, environments—wet tundra of a rich fen type in the inter-drumlin hollows, possibly with the adjacent grassy slopes bearing heliophilous herbs such as *Helianthemum* and *Artemisia*. Very little is known, as yet, of the late-Devensian vegetation of west-central Scotland, but it is now evident that rich fens with *Scorpidium* and *Carex* were widespread during the Allerød oscillation; there are previous records from Garscadden Mains (NS 524712), just West of Glasgow^{1,2}, and from Drymen (NS 490923) near Loch Lomond³.

Table 2 lists the macroscopic plant assemblage sieved from ~500 ml of the clay at North Kerse. The assemblage is mainly, if not entirely, allochthonous. By far the most abundant fragments are of the moss *Racomitrium anuginosum*, an often abundant colonist of freshly exposed rock or mineral soil. *Aulacomnium turgidum* is a common tundra moss, now rare in the Scottish mountains. This species and *Alchemilla vulgaris* agg. have few Quaternary records; the other taxa are well known from the British Quaternary^{2,4}. The material used for radiocarbon dating consisted of fragments of *Racomitrium* with a slight admixture of vascular plant debris. Abundant small fragments of coal were separated by flotation and by manual removal under a dissecting microscope.

The evidence from the three sites may be synthesised as follows:

(1) At Robroyston and Springburn, glacial till on the flanks of drumlins overlies peat deposits dated ~11,650–11,150 b.p. and containing flora indicative of tundra conditions. The till in contact with the organic deposits is unweathered and resembles the typical grey till constituting the drumlins of central Glasgow⁵, till that is commonly regarded as a product of the main Devensian glaciation, thought to have occurred in west-central Scotland broadly between 27,000 and 13,000 b.p.

(2) At Springburn the till underlies peat dated 5,994 ± 50 b.p. and therefore must have been emplaced in its present position by natural processes rather than by the industrial activities of man. Inferentially, the till at Robroyston also was transported to its present position by natural processes.

(3) At North Kerse, the plant detritus dated 11,210 ± 190 b.p. is indicative of tundra conditions. The detritus is overlain by sands and gravels which, on initial investigation, are thought to have been deposited in an aqueous environment.

Possible alternative interpretations of the evidence are these:

(1) The till at Robroyston and Springburn was deposited directly by ice in its present positions in the course of a glaciation that occurred later than ~11,150 b.p. The tundra conditions indicated by the peat deposit at Robroyston, the lower peat at Springburn and the dated organic detritus at North Kerse may represent the onset of colder, glacial conditions in west-central Scotland after a warm episode ~13,000–12,000 b.p. (ref. 6). At North Kerse, the sands and gravels overlying the organic detritus may have been deposited penecontemporaneously with the till in a periglacial regime outside the area of glaciation at Robroyston and Springburn.

(2) After initial deposition by glacier ice, the till at Robroyston and Springburn was moved to its present positions by solifluction processes while contemporaneously the sands and gravels at North Kerse were deposited in a periglacial environment during a cold period that commenced ~11,200 b.p. (cf. the commonly recognised cold episode in Britain between 11,000 and 10,000 b.p.; with average July

Fig. 1 Late-Devensian sedimentary sequences at sites investigated at Robroyston, Springburn and North Kerse, based on borehole records (all three sites) and observations in excavation pits (Robroyston and Springburn). The locations of radiocarbon dated samples within the sequences are indicated by the relevant laboratory numbers. Dates (in yr) are: SRR 696, 11,653 ± 190; BIRM 668, 11,210 ± 150; SRR 697, 11,265 ± 125; SRR 760, 5,994 ± 50; SRR 761, 11,140 ± 110 and SRR 756, 11,210 ± 190.

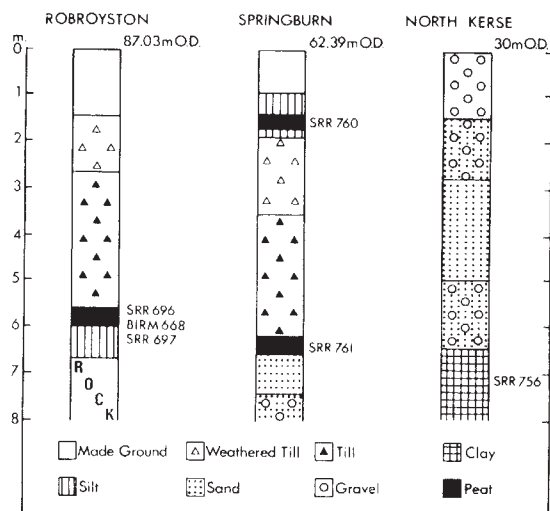


Table 1 Taxa found in the peats at Robroyston and Springburn

Taxa	Sub-till peats	
	Robroyston	Springburn
	per cent	P + S
<i>Betula</i>	2	4
<i>Pinus</i>	+	+
<i>Salix</i>	+	+
Gramineae	2	17
Cyperaceae	91	65
<i>Ericales</i>	1.5	—
<i>Empetrum</i>	—	+
<i>Artemisia</i>	—	+
Caryophyllaceae	—	+
Compositae	+	+
Cruciferae	+	—
<i>Helianthemum</i>	+	+
<i>Myriophyllum alterniflorum</i>	+	—
<i>M. verticillatum</i>	+	—
Ranunculaceae	+	+
Rosaceae	+	7
<i>Thalictrum</i>	+	—
Umbelliferae	—	1.5
<i>Valeriana</i>	—	+
<i>Filicales</i>	+	—
<i>Lycopodium selago</i>	+	—
<i>Selaginella selaginoides</i>	+	—
Unidentified	+	+
$\Sigma P + S$	983	252

+, less than 1 per cent

temperatures below 11 °C suggested by the evidence of Coleoptera⁶.)

The first interpretation must be considered improbable on the grounds that first, in western Glasgow and its environs, grey till constituting drumlins, apparently of the same swarm as those at Robroyston and Springburn, is overlain at a number of locations by marine deposits containing mollusc shells dated 12,600–12,400 b.p. (ref. 7), and second, there is no known evidence to suggest that in west-central Scotland the ice margin of the Loch Lomond Readvance (maximum ~ 10,500 b.p.) extended beyond the southern flank of the Loch Lomond Basin⁸.

Table 2 North Kerse macroscopic plants

Taxa from dry ground habitats		Remains
Mosses	<i>Aulacomnium turgidum</i> (Wahl.)	3 lf
	Schwaegr.	
	<i>Encalypta</i> sp.	1 cl
	<i>Polytrichum alpinum</i> Hedw.	4 st, 6 lf
	<i>Racomitrium lanuginosum</i> (Hedw.) Brid.	> 100 st
Vascular Plants	<i>Alchemilla vulgaris</i> agg.	1 fr
	<i>Salix</i> sp.	1 tw
	<i>Salix</i> cf. <i>herbacea</i> L.	1 lf
Taxa from aquatic, wetland or streamside habitats		
Mosses	<i>Calliergon sarmentosum</i> (Wahl.)	11 st
	Kindb.	
Vascular Plants	<i>Philonotis</i> sp.	2 st
	<i>Carex rostrata</i> Stokes	1 fr
	<i>Menyanthes trifoliata</i> L.	3 se
	<i>Myriophyllum alterniflorum</i> DC	2 fr
	<i>Ranunculus</i> subg. <i>Batrachium</i>	1 fr
	<i>Selaginella selaginoides</i> (L.) Link	1 mg
Taxa unclassified by habitat		
Mosses	Amblystegiaceae	6 st
	<i>Bryum</i> sp.	3 st
	<i>Pohlia</i> sp.	3 st
	<i>Polytrichum</i> sect. <i>Juniperifolia</i> Brid.	1 lf
Vascular Plants	cf. Cruciferae	2 se

lf, leaf; cl, calyptra; st, leafy stems;
fr, fruit; tw, twig; se, seed; mg, megaspore.

The second interpretation lends support to suggestions recently made regarding late-Devensian climatic conditions in Britain, and focuses attention on a number of interesting problems awaiting further investigation. According to Sissons⁹, major periglacial features in Scotland seem to be restricted to the area outside the limit of the Loch Lomond Readvance, and such features are indicative of extremely severe climatic conditions that persisted down to sea level¹⁰. Published evidence of periglacial action in Scotland during the Loch Lomond Readvance consists mainly of solifluction and associated phenomena in the Highland region together with the record of fossil frost wedges in the Midland Valley and, to a lesser extent, in the Southern Uplands. The evidence from Robroyston and Springburn strongly suggests that large scale solifluction on the flanks of drumlins with a major feature of periglacial activity in west-central Scotland in the cold period that commenced ~ 11,000 b.p. and culminated in the Loch Lomond Readvance. Previously, minor solifluction on the slopes of drumlins has been considered probable, but the sites at Robroyston and Springburn provide the first evidence in west-central Scotland of mass movement of till several metres thick. Clearly, because these examples of such movement exist, many more may be discovered in the future. The presence of a datable peat layer below the soliflucted till at Robroyston and Springburn allowed the movement of the till to be deduced. There may be many situations on the flanks of drumlins where adjacent peat layers did not accumulate before the Loch Lomond Readvance, but solifluction of large till masses occurred nevertheless. Such cases will be very difficult to detect. Till-fabric and till-fissure analyses¹¹ may prove effective in some situations. At Robroyston and Springburn preliminary studies of these properties of the till proved inconclusive.

Future research arising from the preliminary study of the three sites may be directed to establishing the vegetational evidence for late-Devensian climatic conditions in west-central Scotland, to assessing the effectiveness of till-fabric and till-fissure analyses in determining mass movement of till on the sides of drumlins, and to considering the modifications of drumlin shapes that occur as the result of till solifluction.

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Changes in net surface charge of hydrous alumina with phosphate adsorption

ADSORPTION of phosphate on hydrous metal oxides is of considerable importance in soil fertility and eutrophication studies. Phosphate is known to be adsorbed on the hydrous oxides displacing aquo ($M-OH_2$) and hydroxo ($M-OH$) ligands; its adsorption makes the oxide surface less positive¹⁻³. I report here on a quantitative study in which phosphate was adsorbed on to hydrous oxides, displacing the ligands, and the simultaneous change in the net surface charge was measured.

The hydrous alumina used has been described elsewhere³ and the pH value at the point of zero charge (PZC) was 9.3. The phosphate adsorbed and the hydroxyl ions released at different

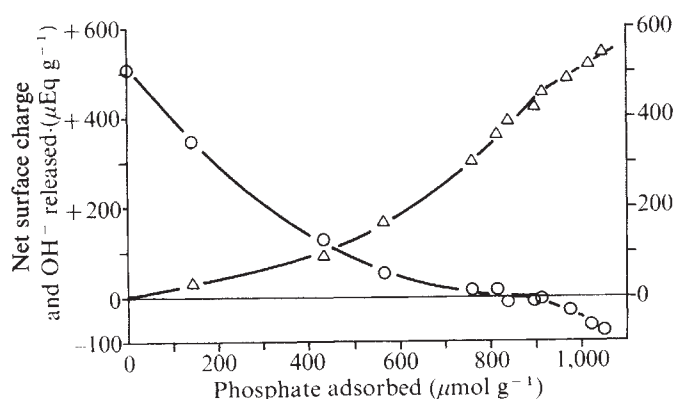


Fig. 1 Phosphate adsorption on hydrous alumina. Net surface charge (○) and OH^- released (△) plotted against P adsorbed.

phosphate concentrations were measured at a constant pH (see ref. 3). The experimental conditions were: pH 5.0; 0.01 mol dm^{-3} NaCl medium; 3 h reaction time; 30 °C; nitrogen atmosphere. The pH was chosen so that phosphate existed in solution as the monovalent ion. The net surface charge (σ) was calculated from the counter ions retained by the oxide in the diffuse double layer (details will be published elsewhere).

The hydroxyl ions released, and the σ values, are plotted against phosphate adsorbed in Fig. 1. Mathematical analysis of the hydroxyl ion curve shows that the differential (dOH^-/dP) increases with increasing phosphate adsorption from 0.11 to a maximum of 1.0 at about 900 $\mu mol\ g^{-1}$ of phosphate adsorbed. At more than about 900 $\mu mol\ g^{-1}$, however, it abruptly decreases to 0.5 and remains constant.

The σ of the sample in the absence of phosphate (σ_0) was 507 $\mu Eq\ g^{-1}$ of positive charge. With increasing phosphate adsorp-

tion, σ decreased to zero, and with further adsorption it became negative.

At pH values more acid than the PZC pH, phosphate is probably adsorbed at the positive sites displacing OH_2 , and at the neutral sites, effectively displacing OH^- (refs 1-3).

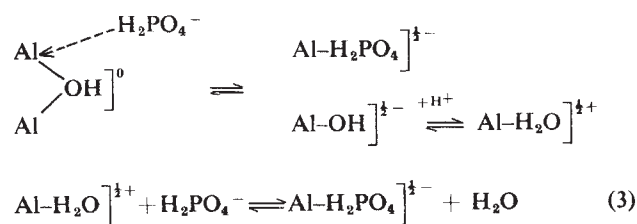


Reaction (1) neutralises the positive charge, whereas reaction (2) leaves the surface charge unaltered.

Because of the greater lability of aquo groups, at low surface saturation more of these are displaced by phosphate, whereas with the increasing saturation the proportion of hydroxo groups released increases³. Therefore, the neutralisation of the positive charge would be initially rapid; this is seen from Fig. 1. There is little change in the σ within the phosphate adsorption range 800-900 $\mu mol\ g^{-1}$, and phosphate is adsorbed mostly by displacing hydroxyl ions, as is evidenced by the dOH^-/dP value of close to 1.

The net charge acquired by the surface per μmol of phosphate adsorbed (ξ') at different adsorption values (Fig. 2) was calculated: the total net charge acquired by the surface, ξ ($\xi = \sigma_0 - \sigma_P$; σ_P = net charge on P added sample) was plotted as a function of phosphate adsorbed, and the equation that gave the best fit was differentiated. The differential curve (Fig. 2) shows that ξ' varies with the type of ligand, OH_2 or OH^- , displaced by phosphate, which in turn varies with the surface saturation. These results suggest that earlier conclusions⁴⁻⁶ that ξ' is independent of surface saturation were based on observations of an experimental artefact. Other researchers have used either a titration method or have relied on separations of the phosphated sample from the matrix, followed by re-equilibration with another indifferent salt solution and extraction of the counter ions using yet another salt solution. It is likely that these methods would result in desorption and/or rearrangement of adsorbed phosphate.

The abrupt change in the slope of Fig. 1 at about 900 $\mu mol\ g^{-1}$ phosphate adsorbed could signify^{3,7} the disruption of hydrous alumina polymers. The fresh surface thus exposed would adsorb phosphate:

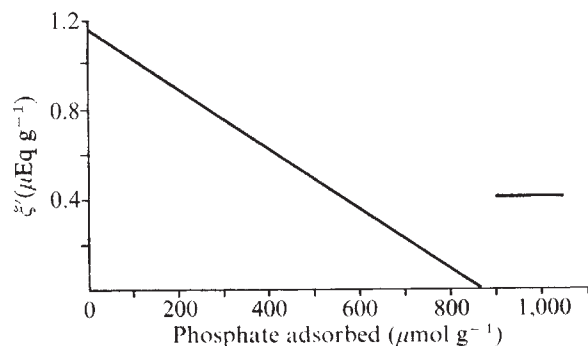


According to reaction (3) a net negative charge of 1 μEq is added for every 2 μmol of phosphate adsorbed. Thus, in the region of high phosphate adsorption ($> 900\ \mu mol\ g^{-1}$) the expected ξ' is 0.5, and the experimental value of 0.41 (Fig. 2) is in reasonable agreement.

Reactions (1) and (2) predict a 1:1 ratio between released OH_2 and ξ values. To test this, ξ values have been plotted against the number of μmol of OH_2 displaced (Fig. 3). The latter values were calculated from the difference between the amount of phosphate adsorbed and hydroxyl ions released. The four observations at $> 900\ \mu mol\ g^{-1}$ of phosphate adsorbed were not used because adsorption was on the new surface in that region, and different reaction paths are involved (3). The observed slope of 1.03 ± 0.05 ($r^2 = 0.989$) agrees with the predicted value of 1.0.

The results presented add further evidence in support of the theory³ on the nature of phosphate adsorption at different surface saturation values.

Fig. 2 Net charge acquired by the surface per unit μmol of phosphate adsorbed (ξ') as a function of phosphate adsorbed.



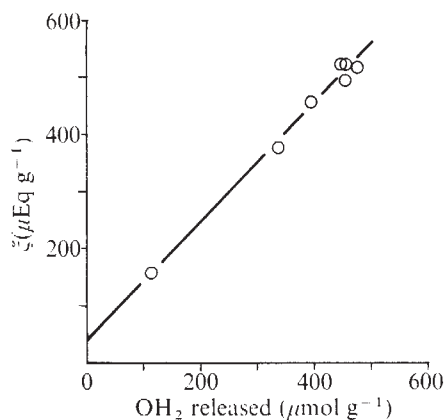


Fig. 3 Relationship between the net charge acquired (ξ) and aquo groups (OH_2) displaced by phosphate. $\xi = 40.46 + 1.03\text{OH}_2$; $r^2 = 0.989$.

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Conformational effects in drag reduction by polymers

An earlier study of drag reduction by polyelectrolyte solutions¹ revealed that the gross flow behaviour varied between extremes, termed types A and B, respectively characteristic of the collapsed and extended macromolecular conformations. Here I show, from examination of all available experiments^{1–5}, that the crossover from type A to type B behaviour occurs at a characteristic conformation, which possibly reflects the role of macromolecular elasticity in the mechanism of drag reduction.

It should be recalled⁵ that drag reduction by dilute polymer solutions in turbulent pipe flow is bounded between two universal asymptotes, that is, the Prandtl–Karman law and the maximum drag reduction asymptote, that envelop a ‘polymeric’ regime in which the observed friction factor relations are approximately linear on coordinates of $f^{-1/2}$ against $\log \text{Re} f^{1/2}$, where f is the friction factor and Re the Reynolds number. The behaviour exhibited by a family of polymer solutions is termed type A when the polymeric regime segments fan outwards from a common onset point on the Prandtl–Karman (Newtonian) line with slopes exceeding the Newtonian value and increasing with increasing polymer concentration, and type B when the polymeric regime segments lie roughly parallel to the Prandtl–Karman line but displaced upward from it, the more so with increasing additive concentration.

The dependence of a drag reduction modulus, κ , on a macromolecular excluded-volume parameter, E , is now sought from

experimental data, these quantities being defined

$$\kappa = (\delta/c^{1/2})M^{1/2}N^{-3/2} \quad (1)$$

$$E = \sigma([\eta]/[\eta]_0)^{1/3} \quad (2)$$

κ is derived from friction factor measurements; δ is the observed Prandtl–Karman slope increment relative to solvent in the polymeric regime of drag reduction while c , M , and N , are respectively the macromolecular concentration, molecular weight, and skeletal links. E is formed from the polymer species chain conformation factor σ and the ratio of actual to theta point intrinsic viscosities, at zero shear, of the macromolecule; as long as the macromolecule remains random-coiling, E is evidently the ratio of actual to unhindered r.m.s. radii of gyration ($R_G/R_{G,0}$). The results are shown in Fig. 1, which shows κ against E on doubly logarithmic coordinates. An abrupt change in κ is observed at $E_x = 6 \pm 1$, defining a characteristic ‘crossover’ conformation. For $E < E_x$, in the region of type A gross flow behaviour, the drag reduction modulus is essentially independent of macromolecular conformation, and the examples in Fig. 1 all display magnitudes of $\kappa_A = 10^{-4}$, typical of random-coiling polymers with polymethylene types of skeletal⁵. For $E > E_x$, in the region of type B gross flow behaviour, the variation of κ with E cannot be ascertained for lack of data, but values of $\kappa_B = 10^{-5}$ are clearly an order of magnitude lower than κ_A . The behaviour described is well illustrated by results for two PAMH polymers in Fig. 1, one of which¹ yields $\kappa \times 10^6 = (61, 60, 61, 5, 5)$ for $E = (3.6, 3.7, 4.9, 6.8, 10.0)$ respectively while the other² (hollow squares) shows a very similar trend. The data^{3,4} for random-coiling PEO and PIB polymers in good as well as in theta solvents both yield κ essentially independent of E over small ranges of $E < E_x$.

A possible physical interpretation of these results involves macromolecular rubber elasticity. E refers to the static (zero shear) conformation of a macromolecule in dilute solution. In

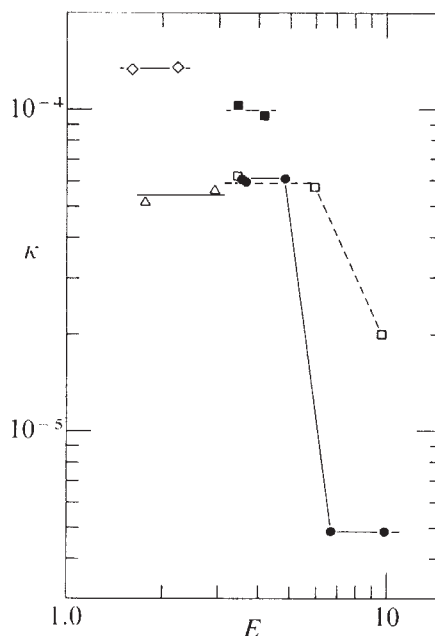


Fig. 1 The dependence of drag reduction slope modulus on macromolecular excluded volume

Symbol	Polymer	σ	Molecular weight ($\times 10^{-3}$)	Solvents	Pipe internal diameter (mm)	Reference
●	PAMH	2.30	15.2	Aq. NaCl, 0.1 M	9.45	1
■	PAMH	2.30	9.2	Aq. NaCl, 0.1 M	10.2	2
□	PAMH	2.30	7.8	Aq. NaCl, 0.2–1.0 M	10.2	2
○	PEO	1.60	0.76	Aq. K_2SO_4 , 0.0–0.6 M	4.57	3
△	PIB	1.82	0.89	Benzene, Cyclohexane	12.9	4

PAMH, Polyacrylamide, partially hydrolysed; PEO, polyethyleneside; PIB, polyisobutylene.

turbulent flow, a macromolecule suffers deformations about its mean conformation and the drag reduction modulus, κ , seems related to rubber elasticity⁵ through a macromolecular chain deformation factor J , defined by

$$J = [(R_G'/R_G) - 1]/\beta^2 \quad (3)$$

where R_G' and R_G respectively refer to deformed and mean conformations, whereas β is a non-dimensional strain rate. This suggests that the data of Fig. 1 arise because the elasticity of a macromolecular random coil is essentially independent of its excluded volume up to a certain conformation, E_x , and then decreases significantly for $E > E_x$. In this context, recent direct measurements of macromolecular extension in a Borda flow⁶ are noteworthy. For two samples of PIB in decalin ($E \approx 3$), it was found that at low strain rates the observed principal axis extension ratio (R_G'/R_G) yielded values of $J \approx 10^{-1}$, of the order of magnitude theoretically predicted⁷. With increasing strain rate, however, the principle extension ratio tended to an asymptote with a maximum value $(R_G'/R_G) \approx 3.5$, with corresponding lower values of $J \approx 10^{-3}$. These experiments independently suggest that there is a characteristic macromolecular extension beyond which elastic deformation of the random-coil becomes difficult. Interestingly, the value of $R_{G,\max}'/R_{G,0}$ (~ 10) for PIB in a Borda flow is of the order of magnitude of our E_x (~ 6) obtained with PAMH during drag reduction, both of the polymers used possessing similar (but not identical) polymethylene skeleta.

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Effect of drag-reducing polymers on pulsation noise of small air bubbles

PREVIOUSLY we have reported¹ that polyethyleneoxide (Polyox) in solution reduced the damping of free oscillations in a semi-circular manometer, whereas polyacrylamide (Separan; also an effective drag-reducer in turbulent flow) did not. Here we report the effect of these additives (Polyox grades WSR 301 and N3000; Separan grades AP 30, AP 273 and MG 200) on the pulsation noise radiated by small air bubbles in water.

The bubbles were produced by passing air through a submerged nozzle in a steel water-tank, $75 \times 60 \times 70$ cm, which was mounted on an expanded polystyrene sheet (5 cm thick), on a concrete base. The compressed air supply consisted of a piston sliding in a cylinder. This was driven by an electric motor through a reduction gear which turned a threaded rod. The air outlet from the cylinder was connected by a flexible PVC tube to a brass nozzle, situated 23 cm below the water level, and mounted in a rubber bush to prevent resonance with the supporting rod. All the nozzles used were 7.5 cm long by 6.4-mm outside diameter, but the inside diameter varied between 2 and 5 mm to give a range of bubble sizes. This arrangement produced bubbles of uniform size at regular intervals. The average bubble size was determined from the volumetric displacement of the piston in the cylinder and the number of bubbles produced in a given time.

The bubble pulsation noise was detected by a hydrophone (Bruel and Kjaer type 8101: uniform frequency range up to 60 kHz), which was fixed at the same depth as the nozzle, but

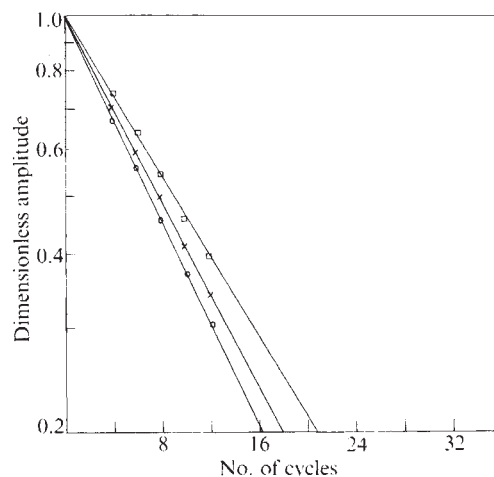


Fig. 1 Time decay of bubble pulsation noise. (Nozzle internal diameter 5 mm; bubble diameter 5.8 mm; f_0 (theoretical) 1,115 Hz; f_0 (experimental) 1,105 Hz). $\times$, water; $\square$, 20 p.p.m. Separan AP 273; $\circ$, 100 p.p.m. Separan AP 273.

could be moved horizontally. The hydrophone was connected to a spectrum analyser (B and K model 2107). The output of the spectrum analyser was connected to a counter-timer (Bradley Electronics 234) used to determine bubble size and frequency of production, a storage oscilloscope (Tektronix 434) used for frequency spectrum and spatial attenuation measurements and an oscillograph (Bell and Howell model 5-137) used for measuring time-decay curves.

Experiments were performed using nozzle diameters of 2, 3, 4 and 5 mm. In each case the additives affected bubble production. We found that at low concentrations, Polyox reduced bubble size (in agreement with previous results² on bubble growth in solutions of WSR 301) whereas Separan tended to increase the size of the bubbles. In both cases the effect was quite small, and the bubble radius quickly became independent of increasing additive concentration. Correspondingly the additives had only a small effect on the natural frequency of the bubble noise.

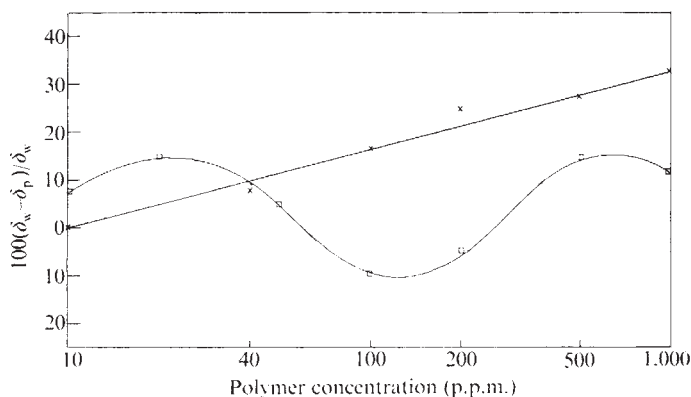
It was also noticed that all additives reduced the radiated sound intensity. This effect has previously been found with noise from turbulent boundary layers³ and from the impact of small spheres⁴ in polyox solutions.

The particular results we report here are concerned with the damping of the bubble noise. When a bubble leaves the nozzle, the emitted sound pulse takes the form⁵

$$P_s = P_0 \exp(-\pi\delta f_0 t) \cos(2\pi f_0 t)$$

where P_s is the instantaneous radiated sound pressure, P_0 is the sound pressure amplitude and f_0 is the natural frequency. The damping constant δ has been predicted theoretically⁶, and

Fig. 2 Variation of reduction in damping with additive concentration. $\times$, Polyox WSR 301; $\square$, Separan AP 273.



the predicted values confirmed experimentally for nitrogen bubbles in water⁷.

To check our system, we first made a comparison with previous work on gas bubbles in water^{6,7}. We found that our values for damping constant and natural frequency were as expected (within $\pm 1\%$). Results for the 5-mm diameter nozzle are shown in Fig. 1. This nozzle size was found to be the most satisfactory in terms of producing a good quality signal with minimum nonlinear distortion and maximum reproducibility. Thus all results presented here were taken with the 5-mm nozzle.

We obtained the time-decay curve by running the oscillograph at a chart speed of 200 cm s^{-1} . The results were analysed by dividing the amplitude at any point by the peak amplitude, and plotting on semi-logarithmic graph paper against time (measured in number of cycles). It was found that all the polymer additives reduced the damping of the emitted sound pulse to some extent, but Separan AP 30 and AP 273 increased the damping at certain concentrations. Representative results for Separan AP 273 at concentrations of 20 and 100 p.p.m. (by weight) are presented in Fig. 1, where they may be compared with the decay curve for water.

These results may be put in a more quantitative form by defining the percentage change in damping constant $100(\delta_w - \delta_p)/\delta_w$, where δ_w is the damping constant in water and δ_p the damping constant with polymer additive. This is plotted against concentration for Polyox WSR 301 and Separan AP 273 in Fig. 2. At low concentrations WSR 301 had little effect. It was only at concentrations measured in hundreds of p.p.m. (which are large compared with amounts needed for turbulent drag reduction) that one noticed appreciable reductions, $\sim 10\%$ – 40% . On the other hand, Separan AP 273 did reduce the damping of the bubble noise at low additive concentrations, but the subsequent dependence of the effect on increasing concentration was rather unusual (see Fig. 2). This oscillatory form of concentration dependence was also observed in the case of Separan AP 30. Possibly this may just reflect the complexity of the bubble noise decay, involving, as it does, energy losses by acoustic radiation, thermal conduction and viscous liquid friction.

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Plasma treatment of coal

COALS of various ranks have been treated in electrical arcs or plasma jets generated from argon, hydrogen, and their mixtures. In most cases the plasmas contained enough energy to heat the coal to temperatures $\geq 1,000^\circ\text{C}$. In this range of temperatures, acetylene formation is thermodynamically favoured, and an argon-hydrogen plasma has been reported to yield a maximum acetylene yield of 74% by weight of coal¹⁻³. Problems are encountered with the electrical arcs because of the instability induced in the arc column by appreciable forced convection⁴. From a practical point of view methane would be the preferred product from coal gasification, and we here report on efforts to find whether a low temperature plasma favoured methane formation.

We applied 50-Hz electrical discharges through 15-cm long coaxial Pyrex tubes, the annular space (1 cm wide) of

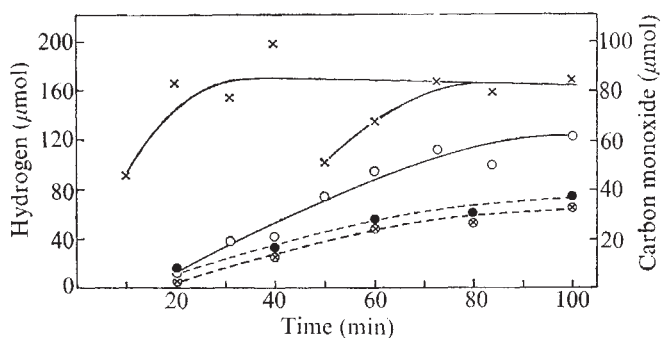


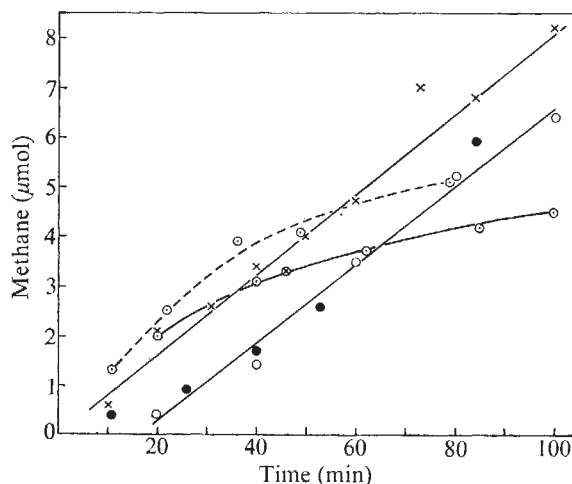
Fig 1 Hydrogen (x) and carbon monoxide (O) yields from a Siemens tube packed with bituminous coal and excited at 68 W. Solid symbols and dotted lines represent the yields from coal + 26 mmHg He.

which was packed with a sample of bituminous coal (76.06% C, 5.48% H, 15.33% O, 1.05% N, and 2.09% S) and degassed at room temperature. The applied voltage was 26 kV and the current through the plasma was measured to be 2.6 mA, which means the power input was $\sim 68\text{ W}$. Gas chromatographic analysis using a 1.2-m long silica gel column at 0°C revealed that the gaseous products pumping out of the plasma zone consisted mainly of hydrogen and carbon monoxide, with small amounts of methane. A 1.2-m long alumina column at 0°C confirmed that acetylene was not present in the products.

Figure 1 shows the yields of hydrogen and carbon monoxide obtained at various time intervals from a sequential operation in which coal was excited, at first alone and then in the presence of 26 mmHg of helium. The influence of the added gas was to decrease the H_2 and CO production considerably. The relatively large amounts of H_2 obtained at the short time intervals appeared to arise from an initial desorption of gases from the coal as a result of the application of the high voltage.

Figure 2 shows the methane formation as a function of the discharge period. Here again the influence of the added helium was to decrease the methane production, except in the long runs ($t > 1\text{ h}$) in which the addition of helium increased the conversion of coal into methane. On the other hand, in the short runs ($t < 0.5\text{ h}$) more methane was produced in experiments in which hydrogen was added to coal which had not been previously treated in a plasma. Table 1 summarises data on the conversion of the carbon in coal

Fig 2 Methane production in a Siemens tube packed with bituminous coal and excited at 68 W. x, O, and $\odot$ for yields from coal, coal + 26 mmHg He, and coal + 26 mmHg H_2 , respectively. Dotted line and solid symbols represent the yields when coal was excited at first in the presence of H_2 and then in He.



into methane. The observed conversions are low for technical purposes, but this is to be expected because of the use of low frequency, low power discharges at low pressures and ordinary temperatures which allowed essentially non-pyrolytic reactions.

Table 1 Conversion of the carbon in bituminous coal into methane

Time (min)	Conversion into methane (mol per cent)		
	Coal	Coal + He	Coal + H ₂
10	12.2	—	17.6
20	12.2	5.2	21.5
40	13.9	8.0	16.2
60	9.1	11.1	11.7
80	12.0	14.8	12.3
100	11.8	14.7	10.8

The electron spin resonance spectrum of the coal subjected to plasma showed a 2.5-fold increase ($\sim 10^{18}$ spins per g) in the spin concentration over an untreated sample suggesting that a free radical mechanism may be involved in the gasification process.

Further work using a flow-system with an argon-hydrogen mixture at atmospheric pressure is in progress.

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pH-dependent heterosis of heavy metal-tolerant and non-tolerant hybrid of the monkey flower *Mimulus guttatus*

ALTHOUGH the toxicity of heavy metals is known to be dependent on pH, no studies of heavy metal tolerance in plants^{1,2} have included it as a variable. We have therefore investigated the direct effect of pH on root growth and root number of two strains of *Mimulus guttatus*, one tolerant and the other not tolerant to heavy metals³. We found that the tolerant strain is also tolerant to acidic pH, whereas the non-tolerant operates better in more neutral pH. The hybrid between the two strains showed pH-dependent heterosis.

The strains used were Napoleon (N) and Cerrig-y-drudion (C). N is tolerant to copper³ and is an annual population collected from a copper mine tailing at a stream edge in the Napoleon locality, Calaveras country in central California. C is sensitive to copper³, and was originally found at the edge of a stream, 2 miles south of the village of Cerrig-y-drudion in North Wales. Seeds were germinated in soil, and clones were established from a single seed and propagated by cuttings. In the following experiment N15 and C16 were used. Although there was much variation between clones (within each population) the range of metal tolerance for N did not overlap with that of C. The F₁ was obtained by crossing ♀♀N with ♂♂C. Only one plant was crossed and different plants were obtained from its seeds.

Cuttings (one node per cutting) of uniform size were obtained from different plants about 0.5 cm below the node. Each cutting was placed in an 11-ml plastic vial filled with a solution of calcium nitrate (0.5 g l⁻¹) with varied pH between 2 and 9.

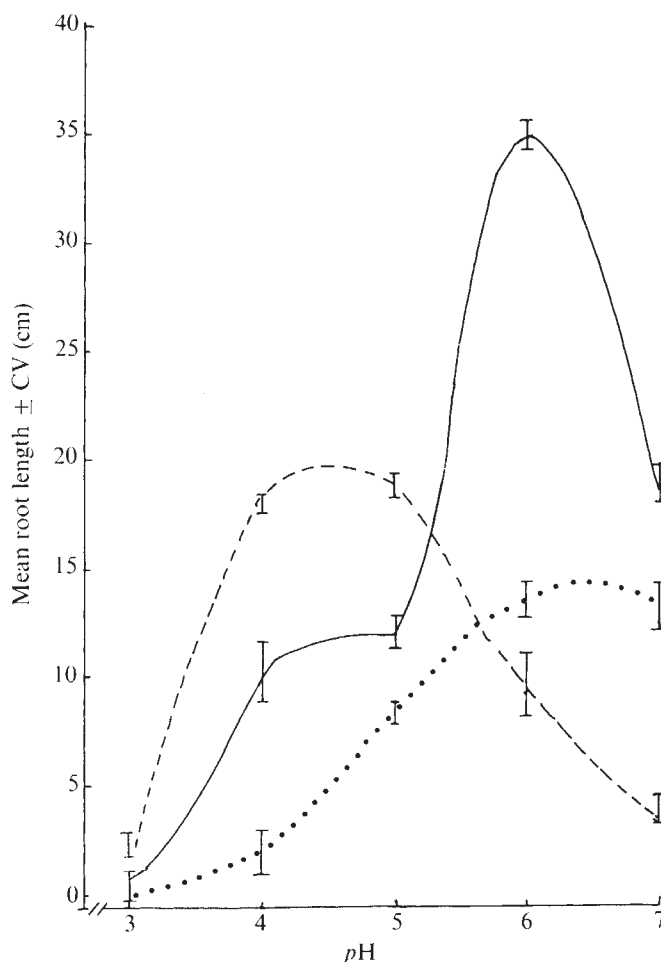


Fig. 1 Relationship between pH and mean root length ($\pm$ coefficient of variation).---, N; ..., C; —, F₁.

Acid pH was obtained with concentrated sulphuric acid, basic pH with sodium hydroxide. Normal calcium nitrate solution gave pH 5. Ten cuttings (replicates) from different plants were used at each pH.

Plastic vials were housed in wooden blocks so that light had no influence on root initiation. Each block was covered with a plastic bag to maintain humidity and placed in a growth chamber operating at a short light cycle (9 h with 25,833.6 lx), with a constant temperature of 20 °C and 55% relative humidity. The number and length of all roots from a given plant were measured. The mean of the 10 plants and coefficient of variation were calculated for each pH and genotype. The coefficient of variation was used to avoid any possible dependence of the variance on the mean.

The effects of different pH on root length are presented in Fig. 1 and Table 1. No cuttings developed any roots at pH 2, 8 and 9. The distribution of root growth between these levels was highly variable (Table 2). Analysis of variance using the actual data or their logarithms gave the same significant results. Using Duncan's multiple range test, it is possible to rank the effect of pH on root length as follows:

pH	3	4	7	5	6
Mean root length	0.60	10.18	12.05	13.05	19.47

where the means at pH 4, 7 and 5 are not significant from each other at $P = 0.05$.

The three genotypes were significantly different from each other in order C (7.52) > N (10.39) > F₁ (15.30). Analysis of variance (Table 2) and Fig. 1 indicate a significant effect of genotype on pH interaction. Strain N preferred the acidic

Table 1 Mean root length for each genotype at five levels of pH

Genotype	3		4		pH 5		6		7	
N	1.33de	(144)	18.11a	(37)	18.82a	(51)	9.74abcde	(136)	3.94bcde	(69)
C	0.00e	—	2.10cde	(98)	8.39abcde	(52)	13.67ab	(75)	13.43abc	(87)
F ₁	0.46e	(172)	10.34abcde	(131)	11.94abcd	(86)	35.00	(74)	18.77a	(81)

Any two means with the same letter are not significantly different from each other at $P = 0.05$ by Duncan's Multiple Range test. Values in parentheses are percentage of coefficients of variation.

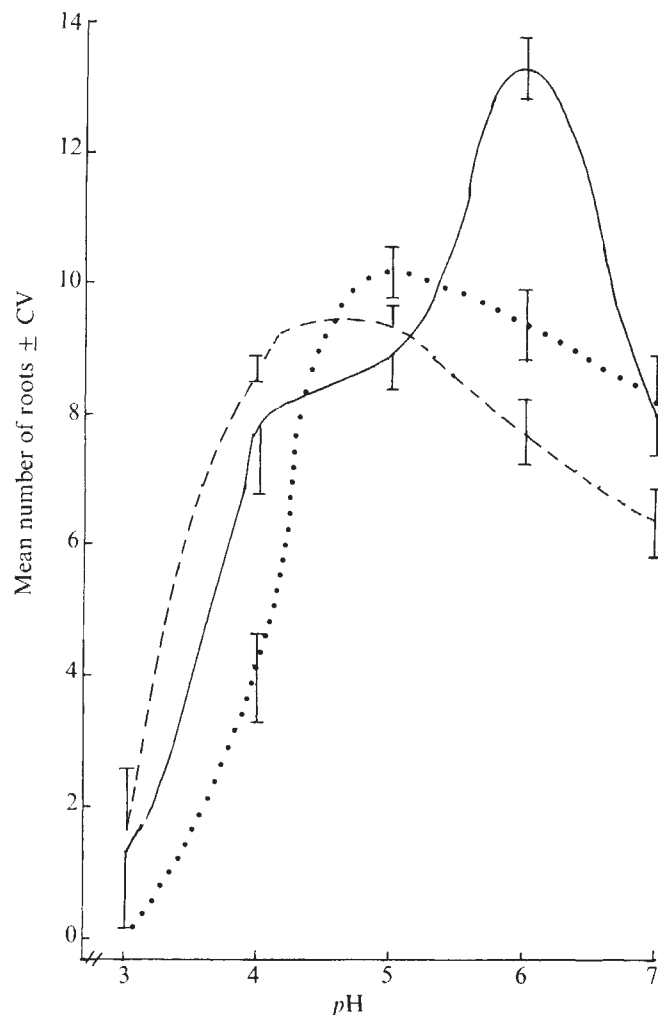


Fig. 2 Relationship between pH and mean number of roots per plant ($\pm$ coefficient of variation). Symbols as in Fig. 1.

solutions with a maximum peak between pH 4 and 5. Maximum root growth for strain C was in the more neutral solutions, between pH 6 and 7.

The F₁ grew better than either parent, with maximum growth at pH 6. Its performance at pH 6 and 7 indicated an overdominance in relation to its parents, whereas between pH 3 and 5 it was intermediate. This suggests two genes operating at different pH levels with different degrees of gene

action. The presence of the shoulder at pH 4 substantiates this hypothesis.

Variation in pH was also found to affect number of roots per plant (Table 2). The average number of roots for the three genotypes could be ranked as follows:

pH	3	4	7	5	6
Mean number of roots	1.0	6.9	7.5	9.5	10.2

where any two adjacent numbers, excluding 1.0, are not significantly different from each other at $P = 0.05$ by Duncan's multiple range test.

These results indicate a gradual significant increase in number of roots from pH 3 to 5. The number of roots at pH 5 was not significantly different from those at pH 6, after which there was a significant decrease at pH 7. Although an analysis of variance did not reveal any effect of the genotype on the number of roots, t values for the difference between N and C at pH 4 ($P = 0.01$) and N and F₁ at pH 6 ($P = 0.05$) were significant.

Phenotypic variation in length and number of roots were calculated within each genotype at each pH. These were expressed as the percentage of the coefficient of variation (Table 1). Analysis of variance on the square of such values indicated no statistical differences between genotypes or pH levels. When the genotypes were compared at each pH, however, the variability for total root growth of both F₁ and C was found to be larger than that of N at pH 4 ($F = 12.15$, $P < 0.01$ and 6.87 , $P < 0.01$, respectively). Whereas at pH 6 the N strain had higher variability than both F₁ and C ($F = 3.43$, $P < 0.05$ and 3.28 , $P < 0.05$, respectively).

The number of roots was less variable. The only significant differences for this trait are between F₁ or C and N at pH 4. F₁ and C variabilities are higher than that of N ($F = 23.97$, $P < 0.01$ and 11.74 , $P < 0.01$ respectively). These results are similar to those obtained for root length at this pH.

It is obvious that N plants are more adapted to growing in lower pH soils than C plants. Whether this is due to direct effect of pH or due to adaptation to heavy metal ions is not known yet and needs to be investigated. (Dr Sheppard informed me that the Californian mines from which the material came are extremely acid compared with North Wales, and he has non-tolerant seeds from a very acid locality.) Undoubtedly, a study of the interaction between pH and heavy metal toxicity is of interest as indicated in Fig. 1, where for example, at pH 5 the N strain has better root growth than the C strain. This performance is reversed at pH 6 and 7 indicating pH-dependent (or conditional) toxicity for heavy metals.

Table 1 shows that pH tolerance of the F₁ is intermediate between that of its parents at pH 3–5 and is overdominant at pH 6–7. Changes in the degree of dominance due to changes in

Table 2 Analysis of variance for length of roots, number of roots, and the ratio between total growth and number of roots at different pH levels

Sources of variation	d.f.	Length of roots	Mean squares	
			Number of roots	Length of roots/number of roots
Replicate(R)	9	101.14	34.73	0.62
pH level(L)	4	1,394.33†	395.86†	9.35†
Genotype(G)	2	774.79†	31.25	2.74*
RL	36	116.56	12.96	0.57
RG	18	135.51	20.76	0.43
LG	8	640.90†	35.42	4.65†
RLG	72	117.40	18.94	0.64

* $P < 0.02$; † $P < 0.01$

the concentrations of heavy metals were also observed³. Parsons⁴ argued that the hybrid advantage is maximum in extreme environments; this is substantiated by the present results at the higher pH levels only. These results also support the theoretical study of environment—dependent heterosis⁵.

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Increased lead ingestion in calcium-deficient monkeys

VOLUNTARY ingestion of lead (lead pica) by children is a puzzling phenomenon. The resulting lead poisoning can lead to painful physical symptoms, mental retardation and brain damage¹⁻⁵. Yet ingestion often persists after toxic symptoms appear if the child has access to lead⁶. We have shown that weanling rats made calcium deficient increased their voluntary ingestion of lead to levels much greater than those of control rats⁷. It was suggested that, because of the metabolic similarity of lead to calcium, ingestion of lead might relieve symptoms of calcium deficiency, attenuate the normal aversive effects of lead ingestion, and thus maintain continued ingestion. Rats, however, are not completely appropriate for an analogy to human lead ingestion because calcium metabolism differs considerably in the two organisms^{8,9}. Rats seem to find lead acetate an aversive taste^{7,10}, whereas humans refer to it as "lead sugar" and report a sweet taste (paper presented at meeting of Midwestern Psychological Association, 1975 by R. W. Henderson and J. Dawley). Furthermore, the diet used with rats has been almost completely deficient in calcium (2 mg

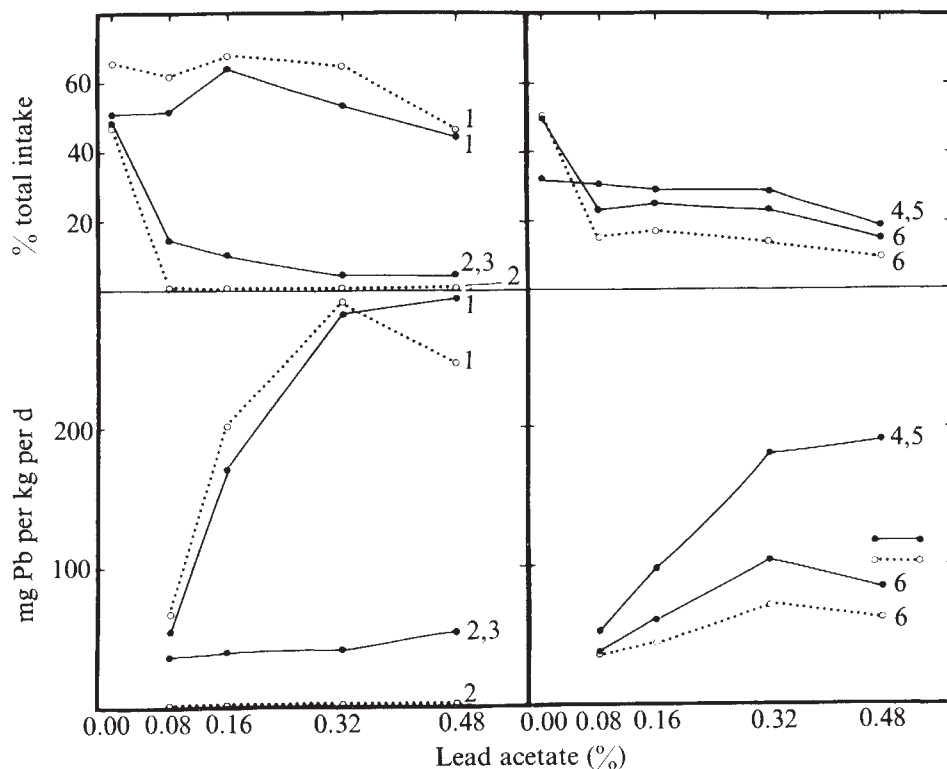
Ca per 100 g) producing severe symptoms of calcium deficiency, which have not been reported in humans with lead pica. If calcium deficiency leads to lead pica in humans, it must be a moderate or subclinical deficiency. We have therefore experimented with rhesus monkeys which were subjected to a moderately deficient diet providing 64% (108 mg Ca per 100 g) of their recommended daily calcium levels¹¹.

Four rhesus monkeys were separated from their mothers shortly after birth and maintained at the University of Wisconsin Primate Laboratory nursery. They were weaned from Similac formula to a Purina monkey chow from which the normally supplemented calcium carbonate and dicalcium phosphate had been removed, leaving 108 mg Ca per 100 g. The monkeys ingested approximately 100 g per day. Six lead ingestion tests were administered—three during deficiency and three during recovery from deficiency when the animals were feeding on normal monkey chow. Two control monkeys that had been weaned to normal chow were available for lead testing only during periods corresponding to the first two drinking tests of the deficient animals and the last drinking test during recovery. Each week the animals were weighed and blood samples were drawn to be analysed by atomic absorption spectrophotometry. Two months after calcium restoration to the deficient animals all animals were given whole body X rays.

Four concentrations of lead acetate (0.08, 0.16, 0.32, and 0.48% weight/volume) were paired with distilled water, and a pair of bottles each containing distilled water was also used. One pair of solutions was presented to an animal each day in a counterbalanced order. The relative positions of lead and distilled water bottles were alternated to control for side preferences, and each of the five pairs of solutions was presented twice. Solutions were presented at 1000 when the animals were being fed and removed at 1800. Distilled water was available overnight, but almost all fluid intake occurred during the hours of the test. For analyses the data were converted to lead acetate intake as a percentage of total intake and to the mg of lead ingested per kg of body weight.

A moderate level of calcium deficiency was produced. With calcium deficiency the body weights of the deficient

Fig. 1 Lead ingestion presented as percentage of total fluid intake (top) and mg of lead ingested per kg per day (bottom) during both calcium deficiency (left) and calcium restoration (right). Numbers at the side of each curve indicate the drinking test. Test 1 began 3 weeks after the deficient animals were weaned to calcium-deficient diet. Tests 2 and 3 began 8 weeks and 10 weeks respectively after the onset of deficiency. Tests 4 and 5 began 5 weeks and 7 weeks respectively after restoration of normal calcium, and test 6 began 24 weeks after calcium restoration. Results of tests 2 and 3 and, subsequently, tests 4 and 5 for the calcium-deficient animals did not differ so each of these pairs of tests is represented by a single mean curve. ○, Calcium deficient; ●, control.



animals decreased below those of the controls, but never went below 2 standard deviations of the mean body weights of normal monkeys at the same age at the Wisconsin Regional Primate Research Center. The X rays revealed growth arrest lines in the long bones of the calcium-deficient animals presumably as a function of calcium deficiency. Because these lines were located 1.5–2.0 cm from the epiphyses, bone growth had resumed after restoration of full calcium levels and, thus, indicated that calcium deficiency had been reversed.

In the first lead drinking test both deficient and control animals ingested large amounts of lead solution at all concentrations, both in terms of percentage intake and in mg per kg of lead ingested. Thus lead acetate seemed to be an initially "good-tasting" substance, in contrast to results from rats^{7,10}. But after the seventh or eighth day of the first drinking test each of the control animals reduced their intake of lead solution from a mean of 273.3 ml d⁻¹ to a mean of 1.4 ml d⁻¹. This low level of intake was maintained by control monkeys throughout the second lead ingestion test. Calcium-deficient animals showed some reduction in lead solution intake from the first to the second drinking test periods (mean 175.7 ml d⁻¹ to 61.0 ml d⁻¹), but they maintained much higher intakes than did controls. Even in a third drinking test the deficient animals maintained higher intake levels (mean 51.0 ml d⁻¹). These data are shown in the left-hand panel of Fig. 1. None of the intake measures on any of the calcium-deficient animals during either drinking tests 2 or 3 overlapped with the data of any control animal from drinking test 2 (Mann-Whitney *U* tests=0).

Blood lead levels were increased in both groups during lead ingestion tests (calcium-deficient mean $\pm$ s.e.m.: baseline, 16.3 $\pm$ 1.9 μ g%; test 1, 228.4 $\pm$ 25.2 μ g%; test 2, 112.3 $\pm$ 57.0 μ g%; control: baseline, 20.5 $\pm$ 1.5 μ g%; test 1, 180.3 $\pm$ 15.7 μ g%; test 2, 96.2 $\pm$ 6.2 μ g%). Evaluation of the X rays of the calcium-deficient monkeys revealed radio-opaque lead deposits at the epiphyses of long bones. The animal evaluated as having the densest lead deposition was the one which had ingested the most lead; the one with the least lead deposition was the one ingesting the least lead. We did not, however, observe any overt symptoms of lead poisoning, possibly because of the temporal spacing of the lead drinking tests.

When normal monkey chow, containing five times the daily minimum recommended calcium levels for young rhesus monkeys, was given to the deficient monkeys, there was a slow, steady increase in body weight and X-ray evidence of renewed growth in long bones. But, the level of ingestion of lead acetate by the previously deficient monkeys remained high, as shown in the right hand panel of Fig. 1. Mean intakes of lead acetate were well above the intakes by control animals in both tests 2 and 6. Even 7 weeks of calcium restoration (test 5) did not eliminate these high levels of ingestion. Six months after calcium restoration (test 6) the intakes of previously deficient and control animals did not differ. The increased lead ingestion of control animals relative to their test 2 levels probably reflects the several months that had passed without exposure to lead, after which the lead solutions again were the novel, "good-tasting" substances they appeared to be in test 1.

In spite of an initially positive taste valence, lead acetate was rejected quickly by normal monkeys. Calcium-deficient monkeys did not appear to recognise the presumed aversive effects of lead ingestion and continued to ingest lead at relatively high levels. This increased ingestion continued until the restoration of normal calcium levels. It seems that the calcium-deficient animals were not sensitive to the negative aspects of lead ingestion, which caused complete cessation of lead ingestion in control animals. This suggests that calcium-deficient animals would not be able to learn a taste aversion to a novel solution paired with the administration of lead acetate. We have shown that control rats

reduced their ingestion of novel solutions paired with intra-gastric intubation of lead acetate, whereas calcium-deficient rats did not reduce lead-associated intake (C.T.S., unpublished). In pilot studies with rhesus monkeys we found reduced intake of lead-associated novel solution on 2 of 4 test days in control monkeys, but no reduction in monkeys that had been previously calcium deficient.

The reason why an organism should voluntarily ingest lead is partially explained. Primates seem to find lead initially "good-tasting" and ingest it in large quantities. But, some post-ingestional, and presumably aversive, aspects of this continued lead ingestion eliminate subsequent lead ingestion in normal monkeys. Calcium-deficient monkeys seem to be insensitive to the presumed aversive aspects of continued lead ingestion and thus do not stop ingesting lead. Given such insensitivity to aversive post-ingestional aspects of lead ingestion, calcium-deficient monkeys, even though recovered from calcium deficiency, might continue to respond to the positive taste valence of lead acetate, by continuing to ingest lead.

Since the calcium-deficient monkeys here suffered only a subclinical calcium deficiency but still ingested lead, children with lead pica may also experience only subclinical calcium deficiency. Almost all attention given to possible nutritional bases for lead pica has focused on iron deficiency, with repeated findings that iron supplementation does not reduce lead pica and that dietary iron seems to be at adequate levels^{12–15}. It would be important to examine children with lead pica for evidence of subclinical calcium deficiency.

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Microvilli and cell swelling

MICROVILLI have various functions connected with an increase in surface area. In the form of brush border of intestinal and renal epithelium, microvilli increase the absorptive capacity of cells. On tumour cells^{1,2} they may have a role in invasiveness³. Their presence on cultured cells⁴, which is apparently controlled by the intracellular concentration of cyclic AMP⁵, increases the surface area so as to facilitate "spreading"⁶ and cytokinesis^{7,8} without concomitant membrane synthesis. In the latter two situations, cell volume remains constant, while the shape changes. The most common situation in which the reverse occurs, that is an increase in surface area accompanying an increase in volume without a change in shape, is during cell swelling. Swelling due to entry of water is caused by (1)

Table 1 Binding of ^{131}I -con A by Lettree cells

Time of inc. (min)	Additions	Control cells ^{131}I -bound (c.p.m.)†	Swollen cells*
0	—	15,000	10,000
5	—	68,000	72,000
10	—	107,000	105,000
5	α -MM	8,000	9,500
0	Cold con A	8,800	7,200
5	Cold con A	54,000	67,000
10	Cold con A	63,000	61,000
5	Cold con A + α -MM	5,100	6,400

α -MM, α -methyl-D-mannoside (30 mM). Concentration of con A was $100 \mu\text{g ml}^{-1}$.

* 1.2 M glycerol for 10 min at 4°C , followed by 0.34% saline for 10 min at 4°C .

† Cells at 4°C incubated with ^{131}I -con A (375,000 c.p.m., 0.04 μg) in final volume of 0.1 ml; stopped by addition of 10 ml saline, spun and pellet assayed. Values are means of three experiments.

exposure of cells to a medium hypotonic relative to that of the cell content or (2) an increase in passive diffusion of cations⁹, such that the Na/K pump cannot maintain an asymmetric intracellular/extracellular distribution. An example of (2) is afforded by the action of Sendai virus on cultured cells; passive permeability is increased¹⁰⁻¹⁴ and cells swell (unpublished results of K.J.M. and C.A.P.). We show here that in both (1) and (2) the increase in surface area can be accounted for by an 'unfolding' of microvilli.

(1) Lettree cells (grown intraperitoneally in mice) were swollen by exposure to either hypotonic buffer (0.01 M sucrose, 1 mM bicarbonate, 2 mM Mg^{2+} , 2 mM Ca^{2+}) (Fig. 1b) or through uptake of glycerol¹⁵ (1.2 M glycerol for 15 min at room temperature) followed by swelling in the presence of 0.25 M sucrose (Fig. 1c). In each case the fully swollen cells were virtually devoid of microvilli in contrast to the highly villated control cells (Fig. 1a). The increase in volume was from approximately $180 \mu\text{m}^3$ to $420 \mu\text{m}^3$ corresponding to an increase in surface area of approximately $120 \mu\text{m}^2$. The number of microvilli on a Lettree cell (approximately 10^3 per cell; mean length $0.46 \mu\text{m}$) increases

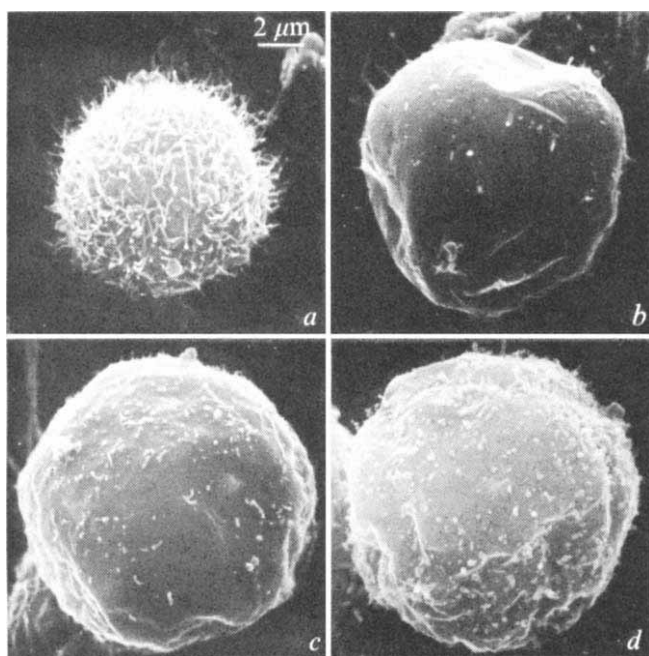


Fig. 1 Scanning electron micrographs of control Lettree cells (a), cells swollen in 0.01 M sucrose (b), glycerol-loaded cells swollen in 0.25 M sucrose (c) and cells after exposure to Sendai virus (d). Cells were fixed with glutaraldehyde at the same osmolarity as the swelling media and processed for scanning electron microscopy as described before⁸.

the surface area by an average of approximately $110 \mu\text{m}^2$. Swollen cells without microvilli bind as much ^{131}I -labelled concanavalin A (con A) as unswollen controls (Tables 1 and 2) in contrast to the effect of microvilli on agglutination by con A (Tables 1 and 2 and ref. 5). Because trypsin treatment causes rounding up of cells and a consequential increase in the number of microvilli it is possible that increased agglutinability (and perhaps stimulation of growth also¹⁶) of trypsin-treated cells^{17,18} is partly due to an increase in the number of microvilli.

(2) Lettree cells (2.6×10^4 per ml) were exposed to Sendai virus (80 haemagglutinating units per ml) at room temperature at a concentration insufficient to cause agglutination¹³. As cells increased in volume (from approximately 700 to $1,200 \mu\text{m}^3$; Coulter size distribution mode value) most of the microvilli were lost again (Fig. 1d).

We conclude that the presence of microvilli enables cells rapidly to increase their volume, and hence to swell, without lysis. Mammalian erythrocytes, which are devoid of microvilli, are capable of a limited amount of swelling because in

Table 2 Agglutination of Lettree cells by con A

Concentration of con A ($\mu\text{g ml}^{-1}$)	Control cells Agglutination index†	Swollen cells*
500	+++++	+
250	+++++	++
150	+++++	+++
100	++++	++
50	++++	++
0	+	+

* As in Table 1 but at room temperature.

† Cells at room temperature mixed with con A and agglutination assessed microscopically: +++++, <10% free cells; ++++, 10-30% free cells; ++, 30-50% free cells; +, 50-70% free cells; 0, >70% free cells.

their normal shape (biconcave disk) the volume is not maximal; once a spherical shape has been adopted, lysis follows. Thus the same concentration of Sendai virus causes lysis of red cells but only swelling of villated cells. During cytolysis of cultured cells in the presence of antibody and complement, an increased cation permeability and cell swelling¹⁹ also precedes lysis. This may explain why cells that are in the late S/G₂ phase of the cell cycle, and hence the most heavily villated^{7,8}, are the least sensitive to immune (or non-immune) cytolysis²⁰.

Since swelling caused by treatment with hypotonic media or with Sendai virus is readily exploitable, the implication that the increase in volume results from an unfolding of microvilli should prove useful in elucidating the role of microvilli (and cyclic nucleotides) in more complex phenomena such as cytokinesis and other situations involving the cell surface.

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Ionic mechanism of the H^+ pump in a snail neurone

THE observed values of pH inside nerve and muscle cells are too high to be explained by a passive distribution of H^+ across the cell membrane^{1–4}. Thus there is believed to be a system, provisionally named a H^+ or proton pump, which transports H^+ out of, or OH^- or HCO_3^- into the cell. In spite of its importance, little is known about this system, perhaps because it cannot easily be studied using radioisotopes, the use of which has yielded much information about the far better known Na pump. One way the H^+ pump can be investigated is by following intracellular pH (pH_i) with a pH -sensitive microelectrode: because a similar Na^+ -sensitive microelectrode can be used to study the Na pump, it is also

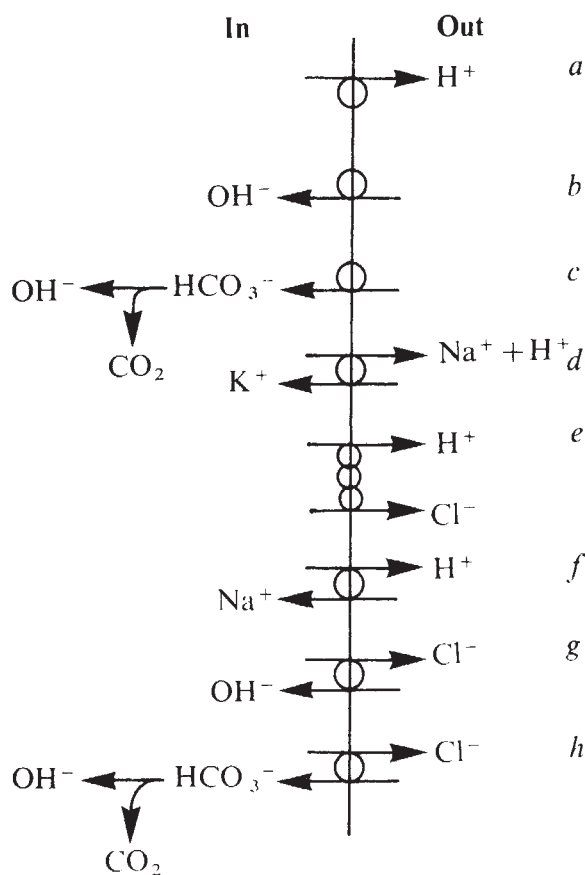


Fig. 1 Diagram showing possible mechanisms for the H^+ or proton pump in cell membranes, excluding any indication of the energy source. *a*, Simple uncoupled H^+ pump; *b*, hydroxyl ion pump; *c*, bicarbonate pump (inside the cell the bicarbonate would give rise to OH^- and CO_2 , the latter being freely diffusible across the cell membrane); *d*, the Na or Na-K pump extruding H^+ as well as Na^+ ; *e*, a loosely coupled HCl pump; *f*, downhill Na^+ influx coupled to H^+ extrusion; *g*, chloride-hydroxyl ion exchange; *h*, chloride-bicarbonate ion exchange.

possible to compare the two pumps in the same preparation using essentially the same methods. Thus the recessed-tip type of pH -sensitive⁵ or Na^+ -sensitive⁶ microelectrode can be used to record pH_i or intracellular Na^+ ($[Na^+]_i$) in cells, such as snail neurones, which are tough enough to permit intracellular iontophoretic injections of acid or Na salts. I report here experiments in which the mechanism of the H^+ pump has been investigated by comparing the response of pH_i to HCl injection with the response of $[Na^+]_i$ to NaCl injection.

Various mechanisms have been proposed^{6,7} to explain the mechanism of the H^+ pumps involved in both pH_i control and acid secretion by the stomach. Some of these mechanisms are outlined in Fig. 1. Schemes *a–d* involve the net transfer of charge across the cell membrane, and would thus be electrogenic⁸, while *f–h* would be electrically neutral. The linked extrusion of H^+ and Cl^- shown in *e* could be electrogenic or neutral. Scheme *d* represents the well known Na pump (or more accurately, the Na-K pump) which might extrude H^+ rather than Na^+ (ref. 9).

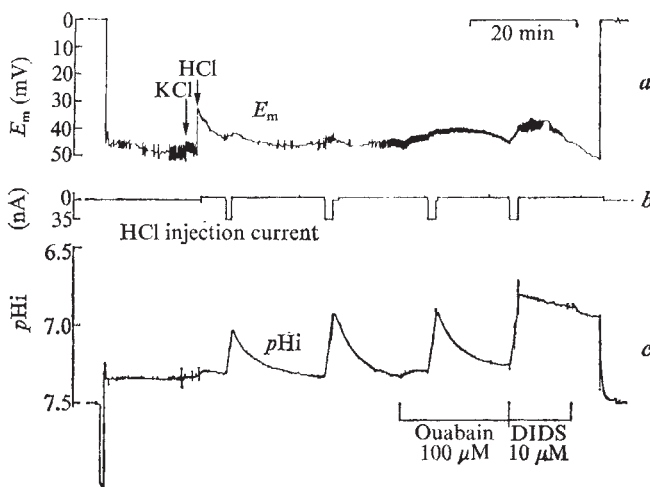


Fig. 2 Recordings showing the effects of HCl injection on E_m and intracellular pH (pH_i) of a snail neurone, and the response to external ouabain and DIDS. *a*, Potential between the E_m microelectrode and a reference electrode in the bath; *b*, iontophoretic injection current; *c*, potential between the E_m and pH microelectrodes. The voltage scale was the same for *a* and *c*. The pH microelectrode was inserted first, then the KCl-filled E_m microelectrode. After about 15 min, the two current-passing electrodes were inserted, and a 5-nA backing current was switched on between them to minimise H^+ leakage from the HCl microelectrodes. HCl injections were made by reversing the iontophoretic current and increasing it to 35 nA for 1 min (first injection) or 90 s (other injections). After the pH_i had recovered from the second injection, ouabain was applied in the superfusate, and after the third injection DIDS was applied, as indicated. Finally the electrodes were withdrawn from the cell.

But evidence from experiments on the effects of ouabain, a specific inhibitor of the Na pump, on muscle pH_i does not support this idea¹⁰. For acid secretion by the stomach, most investigators apparently favour schemes *e* or *h*. The latter has been studied in great detail in red blood cells, where it is known as the "chloride shift", and is an important mechanism in CO_2 carriage by the blood. This anion-exchange in red blood cells is blocked by amino reactive agents¹¹ such as 4,4'-diisothiocyanostilbene-2,2'-disulphonic acid (DIDS). I have therefore tested the effects of both DIDS and ouabain on the Na and H^+ pumps in the snail.

The experiments were done on the largest neurone at the rear of the right pallial ganglion of the common snail, *Helix aspersa*, as described before¹². The exposed suboesophageal ganglia were superfused with a continuously flowing snail Ringer (4 mM KCl, 80 mM NaCl, 7 mM $CaCl_2$, 5 mM $MgCl_2$, 22 mM $NaHCO_3$, 0.1 mM Na_2HPO_4/NaH_2PO_4 (pH 7.5), equilibrated with 2.5% CO_2 and 97.5%

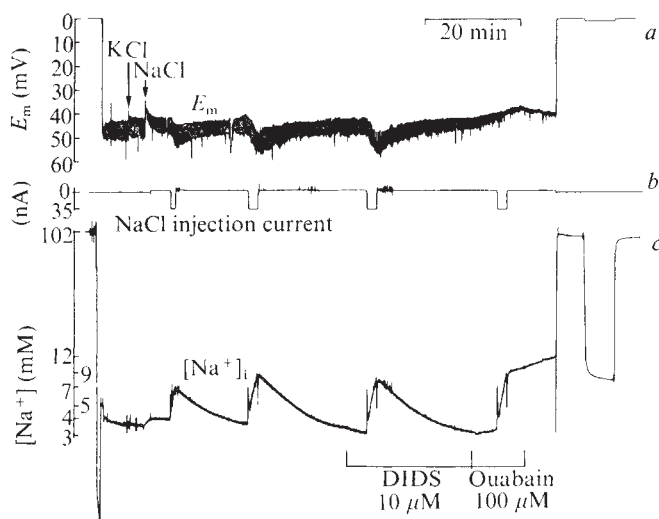


Fig. 3 The effects of NaCl injection on the E_m and $[Na^+]_i$ of a snail neurone, and the response to external DIDS and ouabain. Procedure was as in Fig. 2, except that the duration of the second, third and fourth injections was 2 min, the inhibitors were applied in reverse order and after withdrawal from the cell the electrodes were exposed to a Ringer with 9 mM Na.

O_2). The neurone was penetrated by four microelectrodes: one to measure membrane potential (E_m), one to measure Na^+ , or pH_i , and two for iontophoretic injection, of which one was filled with KCl and the other with NaCl or HCl.

The effects of ouabain and DIDS on the H^+ pump are shown in Fig. 2. During four injections of HCl the pH_i fell. After the first three injections it returned to the pre-injection level during 15 min. The application of the ouabain-containing Ringer caused a small fall in pH_i , presumably because of a slightly lower CO_2 level, but clearly did not block the pH_i recovery. But DIDS, applied at the beginning of the fourth injection, equally clearly did inhibit the pH_i recovery. Other experiments showed that the H^+ pump was very sensitive, 50% inhibition required only about $1 \mu M$ DIDS. Its analogue 4-acetamide-4'-isothiocyanostilbene-2,2'-disulphonic acid (SITS) had the same effect.

Figure 2 also shows that the H^+ pump is not electrogenic: there were only small changes in E_m during pH_i recovery after the HCl injections. This rules out the mechanisms shown in Fig. 1a–d. In contrast, Fig. 3 shows the characteristic E_m increase associated with the electrogenic Na pump. The quantities of NaCl and HCl injected were similar; they were injected into the same cell (in different snail brains) and caused similar changes in the ion equilibrium potentials. Figure 3 also shows that DIDS had no effect on the Na pump while, as expected, ouabain blocked it completely.

In some ways the pH_i and $[Na^+]_i$ responses to HCl or NaCl injection were remarkably similar. The recovery of pH_i was faster, but both recoveries were exponential and repeatable. The effects of ouabain and DIDS were, however, completely different. This clearly rules out any direct connection between the Na and H^+ pumps, as in Fig. 1d.

Since DIDS and SITS are both well established inhibitors of anion exchange in red blood cells, and SITS also blocks the anion-dependent part of the short-circuit current across the turtle bladder¹³, it is tempting to conclude that the H^+ pump in snail neurones is a $Cl^-HCO_3^-$ exchange system, as in Fig. 1h. The finding that the H^+ pump in both squid giant axons¹⁴ and snail neurones¹² is stimulated by CO_2 and HCO_3^- certainly supports this mechanism.

The source of energy for what would be an 'uphill' movement of HCO_3^- into the cell is difficult to identify. The Cl^- equilibrium potential (E_{Cl}) in the neurone studied is normally close to or more negative than E_m , so Cl^- extrusion would also normally be uphill¹⁵. In my experi-

ments E_{Cl} would have been greatly reduced by Cl^- injection, but in other experiments the speed of pH_i recovery was not affected by internal Cl^- levels. It is possible that the anion leaving the cell is not Cl^- , but it is hard to suggest an alternative. It is well established that the Na pump is driven by ATP, but preliminary experiments with metabolic inhibitors suggest that ATP may not be the energy source for the H^+ pump in snail neurones. For example, the uncoupling agent carbonyl cyanide *m*-chlorophenyl hydrazone (CCmP) blocks the Na pump at levels which have little effect on the H^+ pump. On the other hand, Boron and De Weer¹⁴ found that 2 mM cyanide inhibited the H^+ pump in squid giant axons. Perhaps the H^+ pump can tolerate a much lower ATP:ADP ratio than the Na pump.

In conclusion, the results reported here show that there is no connection between the Na and H^+ pumps in snail neurones, and suggest that the H^+ pump is an electroneutral anion-exchange system in which the inward transport of HCO_3^- is coupled to the outward transport of other anions, presumably Cl^- .

I thank Professor H. Passow and Dr H. Fasold for DIDS, Dr Z. Kometiani for helpful discussions and Drs E. Mills and M. Purves for reading the manuscript.

Note added in proof: Recent experiments of mine on the snail neurone H^+ pump suggest that there may be some Na^+H^+ exchange as well as the anion exchange described above. In mammalian cardiac and skeletal muscle SITS has no effect on the H^+ pump (D. Ellis and C. C. Aickin, personal communication), suggesting that the pump mechanism in muscle does not involve anion exchange.

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Role of axoplasmic transport in neurotrophic regulation of muscle end plate acetylcholinesterase

THE mechanism underlying neural control of skeletal muscle acetylcholinesterase (AChE) is not fully understood¹. The experimental evidence points to acetylcholine (ACh)² and nerve-evoked muscle activity³ as the 'trophic' mediators, but neither is sufficient to account for the entire effect of innervation on muscle⁴. It remains to be seen whether factors such as cell-to-cell contact, and/or some regulatory substance(s) released by motor nerves also contribute to the regulation of AChE activity. In turn, the regulatory substance(s) might be conveyed by axoplasmic transport, for blockage of this process causes denervation-like changes without disturbing neurotransmitter release or the consequent muscle contractile activity⁵. We report here a reversible change in the activity of endplate AChE that can be ascribed to a temporary interruption of axoplasmic transport.

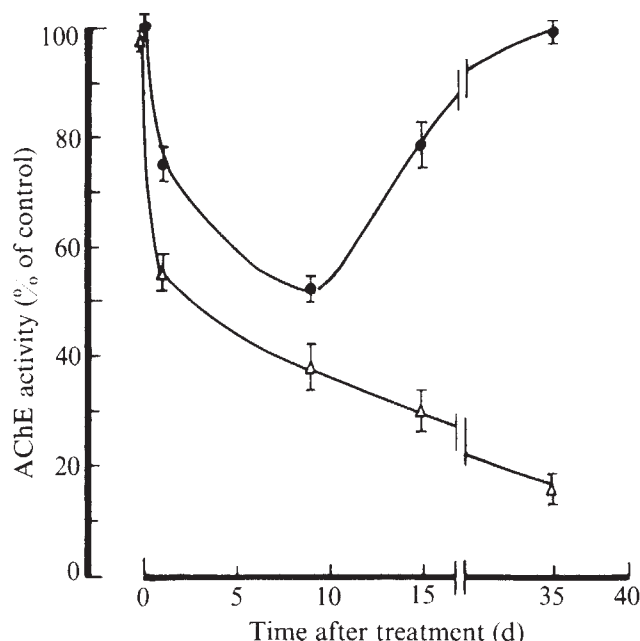


Fig. 1 Effect of axoplasmic transport blockage (●) and denervation (△) on AChE activity, in cat geniohyoid muscle. Muscle regions containing endplates were homogenised 1/50 (w/v) in ice-cold NaCl-Triton buffer, pH 7.3 (1 M NaCl, 0.1% Triton X-100, 0.05 M Tris-HCl, 0.2 mM EDTA). AChE activity as reported here is that portion of the homogenate-dependent ACh hydrolysis that is sensitive to 5 μ M BW 284 C51 dibromide. Activity was measured by a radiochemical procedure in which release of 3 H-acetate from 3 H-ACh iodide (New England Nuclear, 50 mCi mmol $^{-1}$) was quantified by ion-exchange chromatography and scintillation spectrometry⁸. One unit of AChE activity is defined as 1 μ mol 3 H-acetate formed per min. For each assay the final non-collagenous protein concentration¹⁵ was 2–3 mg per ml of homogenate. At the concentrations used, Triton X-100 did not interfere with the AChE assay. Because of changes in the protein content of muscles undergoing atrophy and hypertrophy, the AChE activity could not be correlated with either the initial wet weight, or the particulate protein content of the individual muscle¹⁶, therefore, the results are expressed by comparison of the treated side with the contralateral innervated muscle (control). Each sample was assayed at three concentrations and the activities obtained were proportional to the sample concentration. Each point represents the mean $\pm$ s.e. of three experiments.

Experiments were performed on adult cats, anaesthetised with sodium pentobarbital (36 mg kg $^{-1}$). Both hypoglossal nerves were injected under the epineurium (experimental: 5 μ l of saline, 10 mM colchicine; control: 5 μ l of saline), 5 mm distal to the emergence point of the lateral branch innervating the geniohyoid muscle. Thus surgical damage was avoided since the drug reached the nerve fibres under study only by diffusion⁶. In similar experiments, one of the nerves was transected while the contralateral was left intact. At various times after the operation, the geniohyoid muscles were exposed in order to record their electrical activity and contractile properties. Subsequently they were removed and regions containing most of the endplates (80% of the AChE released by collagenase treatment⁷ came from these regions) were used to assay AChE⁸. Consistent with a previous report⁶, 6–30 d after injection of colchicine, a drug known to interfere with axoplasmic transport⁹, the muscles became hypersensitive to ACh and displayed fibrillation. Nerve conduction and the effective neuromuscular transmission were unaltered. These results were obtained through blockage of axoplasmic transport as induced by the direct action of colchicine on the motor nerve^{6,10}. When applied locally under the epineurium, the drug does not reach the muscle cells. Further, when applied topically on to the muscle at concentrations that interrupt axoplasmic transport, the drug provoked none of the alterations observed

when it was applied to the nerve. Thus colchicine may not necessarily cause denervation-like changes by acting on the muscle membrane¹¹.

Figure 1 illustrates that, after treatment with colchicine, the activity of endplate AChE gradually decreased (days 1–3), reached its lowest level (days 8–10) and returned to control values (days 25–30). In as much as colchicine blocks axoplasmic transport¹⁰, the normal operation of this process seems to be essential for the neurotrophic regulation of endplate AChE activity. Our experiments are insufficient to elucidate the basic mechanism of this dependence. Nevertheless, we can assume that axoplasmic transport supplies some trophic factor to the postneural cell. This rationale is reinforced by findings which suggest the existence of a neurogenic diffusible substance affecting AChE activity of muscle in organ culture¹².

In contrast to the reversible effect of treatment with colchicine, denervation causes a greater enzymatic decay which persists for at least 35 d (Fig. 1). This quantitative difference can be ascribed to: (1) a partial blockage of axoplasmic transport, and/or (2) the regulatory action of other factors, such as muscle contractile activity or ACh itself. Muscle activity has been claimed to be the major factor involved in the control of chick muscle AChE³, but in mammalian muscles it has no such influence¹³. In turn, the role of ACh in AChE regulation is supported by results obtained with botulinum toxin which—apart from inhibiting ACh release—may block the liberation of other neurogenic substances^{2,14}. In view of our results, we conclude that axoplasmic transport is the most important mediator in the trans-synaptic regulation of skeletal muscle endplate AChE activity.

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Three-step yielding of load-clamped mammalian cardiac muscle

FROM mechanical transients of active skeletal muscle in response to length¹ or load^{2,3} perturbations some aspects of the kinetics of crossbridge interactions between actin and myosin filaments have been studied. The analysis of tension transients following rapid length steps has indicated that crossbridges may be the major site of series compliance in active skeletal muscle^{1,4,5}. The use of load steps^{2,6} instead of

length steps has the advantage that any series compliance does not change in length after the step. Yet, in the rapid skeletal muscle load steps cannot be applied sufficiently fast to distinguish the very early events in view of their possible relation to crossbridge interactions⁵. We have analysed length transients after small step increases of load, which did not exceed the isometric tension, during isotonic shortening of afterloaded twitch and tetanic contractions of slow mammalian cardiac muscle at a low temperature, that is cat papillary muscle at 19 °C. Our main conclusion was that motion of attached crossbridges is confined to near 0.80 (twitch) to 1.02 (tetanus) % of total muscle length.

Figure 1 illustrates the effects of a sudden increase of load during isotonic shortening of an afterloaded twitch. There were three distinct phases in the yielding of the muscle during the load clamp. An initial rapid lengthening (phase 1) accompanied the load step itself. The subsequent constant load resulted in a slower lengthening (phase 2) followed by a high velocity lengthening (phase 3). The amplitude of phase 1 varied with the magnitude of the load step (Fig. 1d), but was the same for a given load regardless of the time of the step (Fig. 1c). From the length changes during phase 1 for various loading and unloading steps at any given time, a single uninterrupted force-extension curve was obtained. The length change of phase 2 occurred at constant speed and varied with the magnitude of the imposed load. But analysis of load clamps at various instants before peak shortening (Fig. 1c), of various magnitudes (Fig. 1d) and at various isotonic loading conditions before length change, of phase 2 and the magnitude and speed of phase 2, measured as the length between the end of phase 1 and the length where phase 2 deviated from the tangent to phase 2, did not exceed a particular maximal value. That particular maximal value averaged 0.80 ± 0.05 (s.e.m.; $n=14$ muscles) % of l_{max} . Moderate under- or overdamping of the load step made the transition from phase 1 to 2 respectively slightly more or less distinct than at critical damping but without altering the magnitude and speed of phase 2. The level of constant speed, but not the maximal length change, of phase 2 and the magnitude and speed of phase 3 seemed to be time dependent. To measure the contribution of changing activation in twitch contractions, we performed further experiments on muscles tetanised by trains of high-intensity electrical stimuli at $[Ca^{2+}]$ 10 mM and caffeine 10 mM in the bathing solution¹¹.

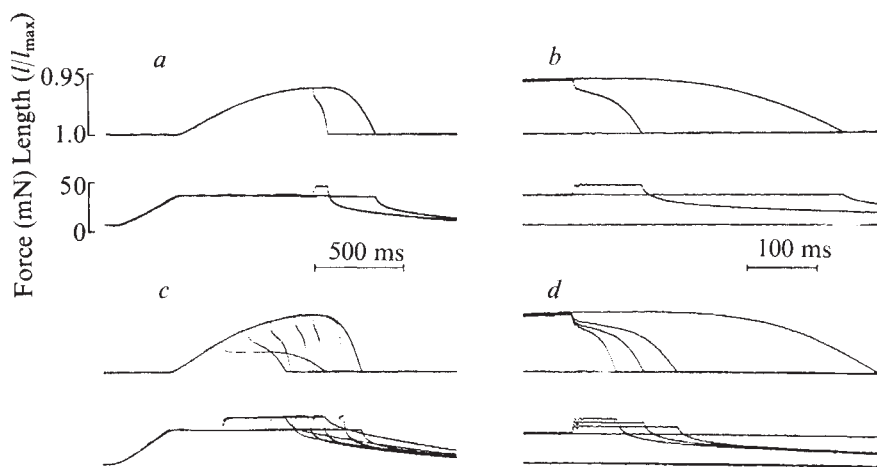
Figure 2 (upper) shows two superimposed load clamps during steady shortening of an afterloaded tetanus. Again,

three distinct lengthening phases were observed. Instantaneous phase 1 varied with the amplitude of the load step. The magnitude of the lengthening during phase 2 was respectively 0.99 and 0.96% for the load clamps *a* and *b*. The mean maximal value was 1.02 ± 0.06 (s.e.m.; $n=10$ muscles) % of l_{max} . Transition to phase 3 occurred earlier in time and at a higher speed as the amplitude of the load step was increased. During a sustained tetanus, phase 3 was followed by a slow recovery (phase 4) without oscillations.

From force-extension analysis, phase 1 was shown to reflect instantaneous undamped series compliance accompanying the load step itself. The nature of series compliance in cardiac muscle is presumably more complex than in skeletal muscle^{1,3,6}, that is crossbridge links between actin and myosin filaments^{1,4}, branching¹², spiral arrangement of the fibres¹³, shearing through Z-lines and intercalated disks¹⁴, and damage of tissue adjacent to the clip¹⁵. We largely bypassed these problems by the conditions of isotonic loading at heavy afterloads, allowing for homogeneous temporal and spatial distribution of force and nearly maximal extension of all non-crossbridge sources of series elasticity before the clamp. Nevertheless, because of the unpredictable relative contribution of crossbridge and extra-crossbridge compliance, phase 1 was not analysed further.

Closer attention was focused on the delayed lengthening of the muscle during the steady-state phase of the load clamp when series elastic elements do not change in length. Analysis of length responses to load clamps in steady-state isotonic conditions also precluded the importance of an unpredictable relation that exists in isometric contractions between sarcomere length and the external length of the preparation and that results from the large amount of connective tissue with uneven distribution of forces¹⁶. Relating overall muscle length to sarcomere length was an assumption underlying the following discussion, and fluctuations of myofilaments about their mean positions within sarcomeres due to fluctuating forces along myofibrils¹⁷ were omitted. Since sarcomere lengths at l_{max} obtained from living cardiac muscle¹⁸ were reported to average $2.23 \mu m$ (ref. 19) and $2.30 \mu m$ (ref. 20) an assumed sarcomere length of $2.25 \mu m$ at l_{max} enables phase 2 to be expressed as an average length change of 9.0 nm per half sarcomere for twitch contractions. Accounting for internal shortening of about 5% of l_{max} resulting from extension of series elastic elements during the isometric phase of the afterloaded contraction¹⁵, yielded a value of 8.6 nm per half sarcomere. Similar calculations for tetanised contractions provided a phase 2

Fig. 1 Length responses of cardiac muscle to sudden increases in load in afterloaded twitch at 19 °C. Although 3-step yielding during load clamps was also observed at higher temperatures (29 °C), the time resolution of the phase 2 lengthening was less satisfactory for further analysis. Length and force gains were the same for all panels. *a*, Slow sweep length and force tracing of load clamp at a time near peak shortening of afterloaded isotonic twitch; control afterloaded contraction is also shown; preload was set at l_{max} , while afterload was augmented to near 70% of isometric conditions so that subsequent isotonic shortening had not proceeded below $0.95l_{max}$ before the load clamp. *b*, Fast sweep tracings of contractions illustrated in *a*; fast sweep begins just before load clamp is applied; lengthening has three distinct phases. *c*, Slow sweep tracings of load clamps at various times during a twitch; fourth clamp from left is the same as in *a*. *d*, Fast sweep tracings of load clamps of various magnitudes at a given time during a twitch; middle clamp is the same as in *b*. All experiments were performed at the initial muscle length l_{max} , that is the length at which active force development of an isometric twitch was maximal. The experimental protocol has been extensively discussed previously⁷⁻⁹. Suitable muscles were selected on the basis of their ratio of resting to total force at l_{max} (refs. 7-9), the mean ratio being 0.11 ± 0.01 (s.e.m.; $n=17$ muscles) at 29 °C and 12 per min. To account for the long term memory of cardiac muscle for loading in preceding contractions¹⁰, all test contractions were separated by a series of at least 6 equally loaded standardised contractions.



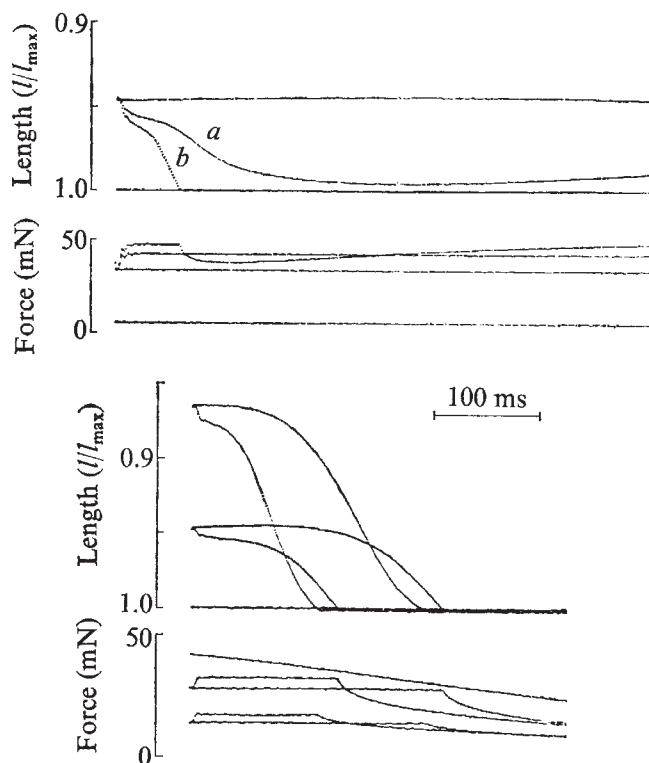


Fig. 2 Upper panel (tetanus): length responses of tetanized cardiac muscle to sudden increases in load (fast sweep); two clamps with different loads were applied during steady shortening of heavily afterloaded tetanus; isotonic shortening had proceeded to about $0.94 l_{max}$ before the clamps; control tetanic contraction is also shown. Tetanic stimulation train was sustained along the whole duration of the record. Lower panel (twitch): fast sweep record of load clamp before and after halving afterload and amplitude of load step during twitch at 19°C . Part of the force tracing of an isometric twitch contraction is also shown.

length change of 10.9 nm per half sarcomere. These values are of the same order of magnitude as the extent of motion ascribed to crossbridges in skeletal muscle¹⁻⁵. We therefore propose that during phase 2 attached crossbridges are forced to rotate back to their first point of attachment, as has been suggested for skeletal muscle^{5,21}. To test the connection of the delayed phase 2 lengthening distance with the extent of a crossbridge stroke, both afterload and load-step magnitude were halved with respect to the control load clamp experiment (Fig. 2, lower). If it is assumed that the number of attached crossbridges is proportionally altered, the extent of phase 2 lengthening should nevertheless be the same in both conditions. The magnitude of phase 2 was not altered indeed ($n=11$ muscles; $P>0.5$) and thus reflects the full range of back rotation of attached crossbridges.

Rapid phase 3 lengthening might then result from forcible breakage of attached crossbridges. The fall of isometric force following completion of phase 3 in afterloaded tetanus was always clearly apparent, even in experiments where steady tetanic shortening at the time of the clamp had not proceeded to lengths below $0.98-0.99 l_{max}$. This suggested that the load step itself or the concomitant lengthening might have detached some load bearing crossbridges and that crossbridges in a less favourable position of attachment could have detached all along the clamp including during phases 1 and 2. Analysis of clamps, which after durations of 2-100 ms were reversed to the original load during phases 1 or 2 in twitch and tetanus, yielded the same conclusion in showing incomplete or delayed recovery to the original shortening tracing. Such crossbridge events could possibly account for the effect of deactivation caused by motion in heart muscle²². Although some crossbridge re-

attachment might have occurred from the onset of the load clamp², the far delayed recovery phase 4 during tetanus would predict reattaching during phases 1, 2 and 3 proceeding at a relatively much slower rate than detaching.

Another possible explanation is that of a lengthening pattern following the load step but not uniform with respect to time in all segments along the length of the preparation during a twitch, with lengthening in some portions of the fibre and actual shortening in others^{1,6,23}. Phase 2 could then result from the delayed relaxation of those sarcomeres exhibiting the longest duration of mechanical activity. The three step yielding during tetanus under homogeneous temporal and spatial activation rules out the contribution of such longitudinal inhomogeneity of the duration of activity.

In conclusion, using the slow contraction mode of mammalian cardiac muscle at low temperature, we have analysed the delayed lengthening after load perturbations and we propose that in cardiac muscle, as in skeletal muscle¹, force is generated by the total number of bridges attached in parallel between myosin and actin, pivoting about their attachment with a turnover of 8.6-10.9 nm per half sarcomere, thus producing a force by extending the more compliant portion of the bridges by the same amount.

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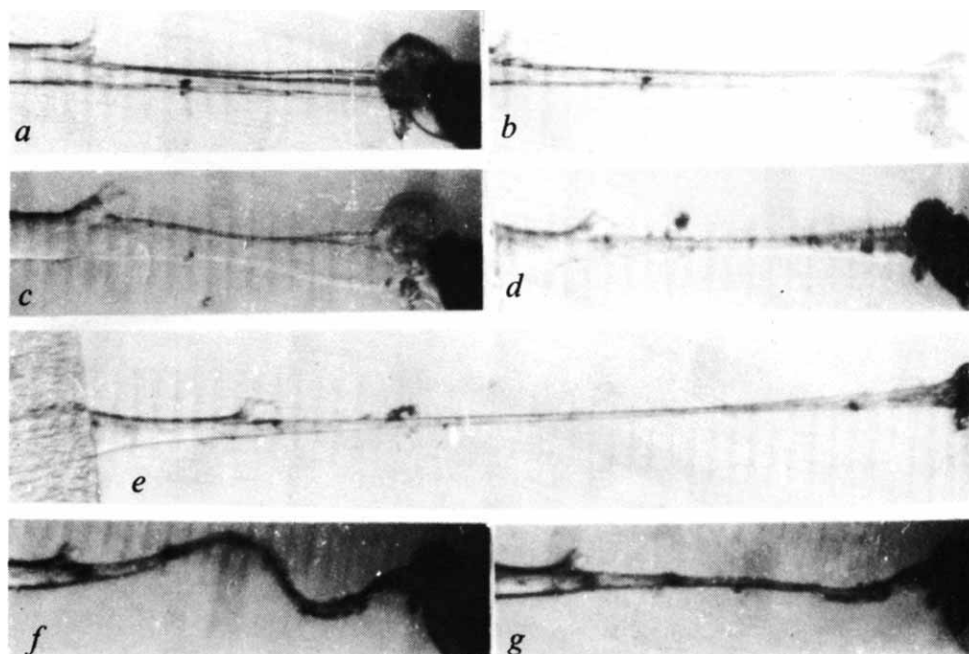
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New elastic protein from muscle

It has long been assumed that an elastic component other than extracellular collagen fibres is present in muscle fibres to explain their elastic properties, especially during passive stretch^{1,2}. The presence of such an elastic component has been demonstrated in skinned fibres of frog skeletal muscle^{3,4}. Furthermore, it has been observed that myofibrils after the removal of myosin are reversibly extended, indicating their structural continuity⁵. The chemical entity responsible for the intracellular elasticity of muscle has, however, remained obscure. When rabbit myofibrils were thoroughly extracted with salt solutions such as 0.6 M KI, it was noticed that remaining Z lines still maintained their continuity, although nothing could be seen between the adjacent Z lines under a phase microscope (compare ref. 6). Starting from this observation, we have been able to isolate an elastic protein from myofibrils which is clearly distinguishable from elastin or collagen.

Fig. 1 Passive stretch of 'ghost' skinned fibres. *a*, Skinned fibres of sartorius muscle of the bullfrog in liquid paraffin, 20° C; *b*, stretched skinned fibres; *c*, skinned fibres treated with an alkaline solution (1 M KI, 0.1 M Na₂S₂O₃, pH 11.5); *d*, after 20 h in the alkaline solution, fibres were washed with Endo's solution (107 mM K-methanesulphonate, 4 mM MgSO₄, 4 mM ATP, 2 mM EGTA, 20 mM Tris-maleate, pH 6.8); *e*, the same, stretched; *f*, the same immediately after release; *g*, the same, 1 s after release.



Myofibrils were prepared from rabbit psoas muscle according to Perry and Grey⁷. It turned out, however, that the myofibrils contained broken muscle fibre fragments together with free myofibrils and it was necessary to separate them by sucrose density gradient centrifugation (see ref. 8). Light and electron microscopic examinations revealed that myofibrils were free of fibre fragments and connective tissues and fibre fragments were contaminated a little by free myofibrils. Both preparations were exhaustively extracted with Hasselbach-Schneider solution and water, and then with 0.6 M KI (ref. 9). After the residues were extracted with 1 N acetic acid overnight, they were washed with water and extracted in 0.1 N NaOH for several hours at room temperature. The suspension was centrifuged to remove a yellowish supernatant. After neutralisation the residue was treated with hot phenol to remove some glycoproteins. The phenol-insoluble material was washed with water. Approximately 50 mg of protein was obtained from pure myofibrils (1 g protein). A somewhat larger amount was present in fibre fragments (70 mg per 1 g protein). The final product was not soluble in 1% sodium dodecyl sul-

phate, and therefore, no protein band was detected in a gel electrophoresis.

As the phenol-treated residue was washed with water, the material became adhesive and finally formed a lump. It was possible to prepare a thread of the protein by cutting out the lump. Stretched to 130% of the initial length, the thread was at a tension of as much as 2 kg cm⁻². Examination of the tension-length relationship at different temperatures showed that the thread had rubber-like elasticity (compare ref. 4). The Young's elastic modulus was about 4 × 10⁶ dyne cm⁻², a magnitude similar to that of unvulcanised rubber. The protein is therefore an elastic protein.

The amino acid composition of the muscle elastic protein is given in Table 1. The proteins from myofibrils and fibre fragments were similar to each other in amino acid composition. It is of some interest that the glycine content is much smaller than that of collagen, elastin¹⁰ or resilin¹¹, which are well known as elastic proteins (see ref. 12). The muscle elastic protein contained a small amount of hydroxyproline, which is abundant in collagen or elastin (cf. ref. 12). The amino acid composition is similar to that of non-collagenous reticulin, an extracellular glycoprotein of the basement membranes¹³, which is shown to be present in the endomysium of muscle¹⁴. There are distinct differences between the two proteins in hydroxyproline, cystine/2 and leucine content. The reticulin is soluble in 0.05 N NaOH¹³. Furthermore, the elastic protein from myofibrils and fibre fragments contained only 0.5–1.0% of neutral sugars in comparison with 4% of reticulin¹³.

The localisation of the elastic protein in myofibrils is still obscure, but it seems that it is responsible for their continuity. After extraction of a skinned fibre of frog muscle with an alkaline solution of pH 11.5, the elastic structure seemed to remain intact, although other structures visible under the microscope disappeared. It developed tension when stretched and behaved as an elastic body (Fig. 1). Treatment with 0.1 N NaOH resulted in a considerable decrease in tension generation, suggesting that the elastic properties had been damaged.

The muscle elastic protein makes at least some contribution to the intracellular elasticity of muscle fibre. The relationship between the elastic protein and reticulin or collagen at the myotendinous junction is of much interest from the viewpoint of tension transmission.

Table 1 Amino acid composition of elastic protein from pure myofibrils and fibre fragments of rabbit psoas muscle*

	Pure myofibrils	Fibre fragments
Hypro	7	12
Asp	95	92
Thr	61	59
Ser	61	60
Glu	130	128
Pro	63	65
Gly	89	104
Ala	82	84
Cys/2	2	2
Val	62	60
Met	24	23
Ile	53	52
Leu	73	70
Tyr	30	28
Phe	29	29
Lys	66	61
His	16	15
Arg	57	56

*Number of residues per 1,000 residues

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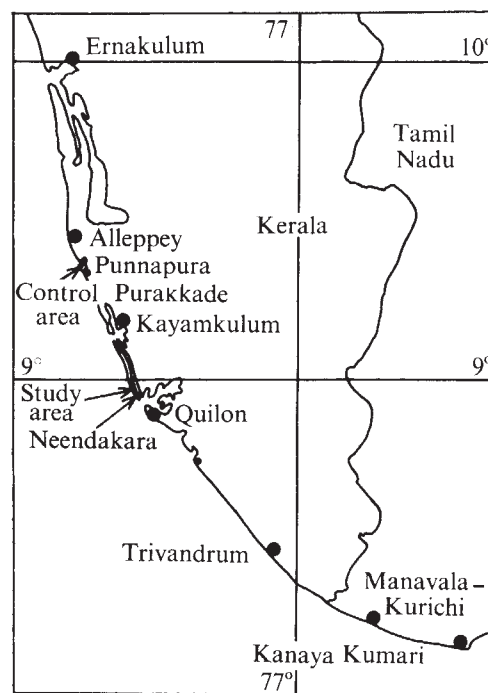


Fig. 1 Map showing the study and control areas.

in the area with high background radiation. The area surveyed was the southernmost one-fifth of the Chavara-Neendakara strip (Fig. 1). In the thatched huts which constitute 75% of all households, the exposure risk is 1,500–3,000 mr. yr⁻¹, and personal exposure, as measured by calcium fluoride dosimeters, closely parallels the exposure risk in the households. The control area consisted of the Purakkade-Punnapura villages, with a background radiation of approximately 100 mr. yr⁻¹ (ref. 7). Households were visited repeatedly to ensure examination of all members. Only gross abnormalities evident on clinical examination were recorded. Cytogenetic abnormalities were scored blind on slides prepared from 64-h micro-blood cultures¹¹.

Demographic and epidemiological details are reported elsewhere⁹. Briefly, the study and control populations were similar in age and sex structure, ethnic composition, living and diet habits, social customs, economic status and occupation. The mean age of all women was 30.3 ± 7.09 yr and 30.12 ± 7.04 yr in the study and control populations, respectively. The frequency distribution by age of women at the birth of the last child was similar in the two populations. The mean family size was 6.2 in the study and 6.4 in the control population. Rate of consanguinity was 12% in the study population and 15% in the control. Abortion rate per 1,000 pregnancies was 109 and 64.5, respectively, in study and control populations ($P > 0.05$), based on a random survey of 57 couples in the study area and 68 couples in the control area.

Table 1 Prevalence of severe mental retardation*

Type	Study population (12,918)		Control population (5,938)	
	Total	Per 1,000	Total	Per 1,000
Genetic				
Down's syndrome	12	0.93	0	0
SMR with physical abnormalities	12	0.93	1	0.17
Idiopathic	11	0.90	3	0.50
Acquired (Perinatal and postnatal)	6	0.46	3	0.50
TOTAL	41	3.1	7	1.16

*The definition of severe mental retardation (SMR) is according to the WHO Classification of 1968. Figures in parentheses indicate the total number of persons surveyed.

Down's syndrome and related abnormalities in an area of high background radiation in coastal Kerala

BOTH point mutations and structural aberrations of chromosomes are induced by ionising radiations, causing genetic variation and abnormalities in man and other organisms. The mutagenic effects are dose dependent and in *Drosophila* a linear relationship between dose and mutation rate has been shown for doses up to 5 R (ref. 1). Although man accumulates approximately 5 R of radiation from the environment in 30 yr of reproductive life, it is not known whether this is of any radiobiological consequence². Nor is it known whether in man there is a threshold phenomenon at low doses (several hundred or thousand mr. per year), although there is greater repair of mutational or pre-mutational damage after low-dose irradiation³. In a coastal area of Kerala, South India, the background radiation is 1,500–3,000 mr. yr⁻¹ due to the presence of thorium-containing monazite mineral in the soil^{4–7} (Fig. 1). A survey of the rat population in this area with respect to several measurable and non-measurable traits and of humans with regard to dermatoglyphics and demographic data such as fertility index, sex ratio and infant mortality rate revealed no mutational effects^{4,7,8}. During an epidemiological study of nodular lesions of the thyroid in this area⁹, we noticed an apparently high prevalence of Down's syndrome and other forms of severe mental retardation¹⁰. We therefore made a house-to-house survey of developmental abnormalities in this area and in a comparable control area without high background radiation⁷ (Fig. 1). We also determined the frequency of chromosome aberrations in a sample of the normal population living in the study and control areas. The observations we report here support the view that radiation-induced genetic anomalies occur with above average frequency in the population living

Severe mental retardation was the commonest developmental abnormality encountered in the study area (Table 1). Clinically, 85% of the abnormalities detected in the study population were "genetic" in origin¹⁰, compared with the 56% in the control population. The frequency of acquired retardation was comparable in the two populations. Prevalence of Down's syndrome was 0.93 per 1,000 in the study population, accounting for a third of the genetic retardations observed, whereas no cases were detected in the control population.

Chromatid and chromosome aberrations were scored in 1,705 metaphases from 46 subjects and in 1,547 metaphases from 39 individuals in the study and control areas, respectively. The mean frequency of aberrations was higher in the study area (chromatid, 3.0 ± 4.6 compared with 1.2 ± 1.9 ; chromosome, 1.9 ± 3.1 compared with 0.2 ± 0.6). Considering the high variance of mean aberration number, a χ^2 test was applied by grouping the data as shown in Table 2. This revealed no significant difference in chromatid aberrations between the two groups

Table 2 Frequency of cytogenetic aberrations

% Aberrations	Chromatid		Chromosome	
	Study	Control	Study	Control
0	20	23	24	36
1-3	12	11	13	3
>3	14	5	9	0
	$P > 0.05$		$P < 0.01$	

Figures in columns 2-5 give numbers of individuals.

*These were mostly deletions (acentric fragments and minutiae), with few rings and dicentrics.

($P > 0.05$), while the difference in the chromosome aberrations was significant ($P < 0.01$).

The observed frequency of Down's syndrome in the study population (1:1,076) was higher than in the control population ($P < 0.05$), and was significant for the following reasons. (1) Although no data are available on the population frequency of Down's syndrome in India, there are figures for the Eastern counties of England, 1:10,000; London, 1:3,000; Germany, 1:7,000; Denmark, 1:4,000; Australia, 1:2,083, and North-east Scotland, 1:2,000¹²⁻¹³. The frequency in Kerala is higher than in any of those (except the Shetland islands, for which the value

Down's syndrome born to mothers more than 30 yr old in the study area (91%) with that among 615 cases documented in India (51%)¹⁸.

Epidemiological and experimental evidence is accumulating which suggests an association between low dose irradiation of older mothers and non-disjunctional disorders in the offspring¹⁷⁻¹⁹. The maternal age-dependence suggests that the damaging event accelerates oocyte ageing and causes primary trisomy rather than translocation trisomy which is known to be independent of maternal age¹⁷. These observations agree remarkably well with our results. In 11 of 12 cases of Down's syndrome maternal age was greater than 30 yr and six of the cases who were successfully karyotyped had trisomy 21. The higher prevalence of chromosome aberrations in apparently normal subjects from the radiation area also supports the view that environmental radiation may be the cause of the genetic damage in the population. Similar cytogenetic findings have been reported from a Brazilian population living in an area of high natural radioactivity²⁰.

The prevalence of severe mental retardation of genetic origin was four times higher in the study than in the control population, whereas that due to acquired perinatal and postnatal causes is comparable. The nature of the genetic defect remains undetermined in most of these patients, except in the group with Down's syndrome, where trisomy 21 was demonstrated by karyotyping. Mental retardation was associated with different physical abnormalities in 12 patients in the study area. Although karyotyping was not done in these patients, chromosome imbalance is now recognised to be important in the genesis of such syndromes^{21,22}. Kirman suggested that a grade of genetic defect intermediate between, on one hand, gross and visible chromosome errors, and, on the other hand, point mutations involving structural changes in the chromosomes, such as inversions and crossovers, may cause idiopathic mental retardation²³. In view of these possibilities, radiation-induced genetic damage seems likely to be responsible for the high prevalence of mental retardation in the area we studied.

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Table 3 Frequency of Down's syndrome distributed by maternal age at conception in study area

Maternal age (yr)	No. of females	% of all females	No. of cases	Frequency
20-29	862	13.6	1	1:862
30-39	729	11.5	9	1:81
40-49	532	8.4	2	1:266

Comparative figures for the distribution of females in the control population were: 20-29-yr group, 478 (16.5%); 30-39-yr group, 369 (12.7%); 40-49-yr group, 264 (9.1%). There was no case of Down's syndrome in this population.

is 1:714)¹³ although infant mortality is four to five times greater than in these areas. (2) The frequency of Down's syndrome among births in India as a whole is 1:1,215 (ref. 14). In Madras, South India where the people are ethnically closer to those in Kerala, the incidence is lower (1:3,833). The frequency in the general population is expected to be lower than this because of the higher mortality among patients with Down's syndrome¹⁵ while in Kerala the frequency is higher. (3) Most striking is the higher frequency of cases of Down's syndrome born to mothers aged 30-39 yr (Table 3). This figure of 1:81 can be compared with 1:880 and 1:290 for maternal ages of 30-34 yr and 35-39 yr, respectively, calculated by Penrose and Smith¹². (4) The increase is also evident in a comparison of the proportion of cases of

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Modulation of the synthesis *in vitro* of a hormone-induced protein by transfer RNA

WE have shown that transfer RNAs (tRNAs) and the enzymes which modify their structure at the macromolecular level (tRNA methyltransferases) are under hormonal control in the target tissue¹⁻³. Oestrogen-induced, cell-specific protein synthesis in immature chick oviducts has been extensively studied⁴⁻⁶. Oestrogen induces cytodifferentiation of the primitive oviduct and the consequent synthesis by the tubular gland cells of ovalbumin, conalbumin, ovomucoid and lysozyme⁷. Continuous presence of oestrogen is required for the sustained synthesis of these proteins in immature chicks; withdrawal of oestrogen is accompanied by a gradual decline in cell-specific protein synthesis⁸. The tubular gland cells in the oviduct magnum, however, continue synthesising non-secretory proteins. Readministration of oestrogen to chicks after withdrawal (secondary stimulation) results in the restoration of the synthesis of cell-specific proteins without concomitant need for DNA synthesis. We report here that in an *in vitro* tRNA-dependent protein-synthesising system from Ehrlich ascites cells, stimulation of the translation of ovalbumin mRNA was significantly enhanced by tRNA extracted from the oviduct of oestrogen-stimulated immature chicks or from the oviduct of laying hens compared with tRNA from oviducts of withdrawn chicks. Therefore, the efficiency of synthesis *in vitro* of a hormone-induced protein can be regulated by tRNA.

We had found previously that oestrogen-induced synthesis of ovalbumin can be enhanced by tRNA isolated from the oviduct of the laying hen, when it is added to oviduct explants of immature chicks⁸. To study the mechanism of enhancement of ovalbumin synthesis by tRNA we developed an *in vitro* protein-synthesising system which is completely tRNA dependent⁹. Such a system was essential

for studying the contribution of tRNAs from different sources to the efficiency and fidelity of translation of a specific messenger RNA (mRNA); otherwise the residual endogenous tRNA can mask any specific effect¹⁰.

Figure 1 shows the effect of tRNA from three different sources on amino acid incorporation. It is evident that tRNA from the oviducts of oestrogen-stimulated chicks or from oviducts of laying hens produced an enhanced stimulation of amino acid incorporation in the presence of ovalbumin mRNA (*b*, upper curve). On the other hand, the tRNAs from the three sources were equally effective in stimulating amino acid incorporation with residual endogenous mRNA.

The efficiency of translation of oviduct mRNA was also assayed by the extent of synthesis of ovalbumin and the results are presented in Fig. 2. Ovalbumin, precipitable with specific antisera, was synthesised to a 70% greater extent in the presence of tRNA from oestrogen-stimulated chicks than in the presence of tRNA from chicks that had not received secondary oestrogen stimulation.

The identity of the polypeptides precipitated by antisera was confirmed by gel coelectrophoresis with authentic markers (Fig. 3). The faster moving components are probably nascent, incomplete ovalbumin precursors. The experiments were reproducibly repeated with three different preparations of tRNA, isolated from different batches of chicks, using separate preparations of the protein-synthesising system from Ehrlich ascites cells. There were some variations in the activity of the protein-synthesising systems. In different experiments, however, ovalbumin mRNA translation was 50–75% greater by oviduct tRNA from oestrogen-stimulated chicks than from withdrawn chicks. For reproducible results it is essential to use protein-synthesising systems which depend completely on the presence of exogenous tRNA.

To rule out the possibility that the relative inefficiency of oviduct tRNA from withdrawn chicks in the synthesis of ovalbumin stems from some unknown artefact of the isolation, its effectiveness in the translation of another mRNA was tested. In the efficiency of translation of rabbit

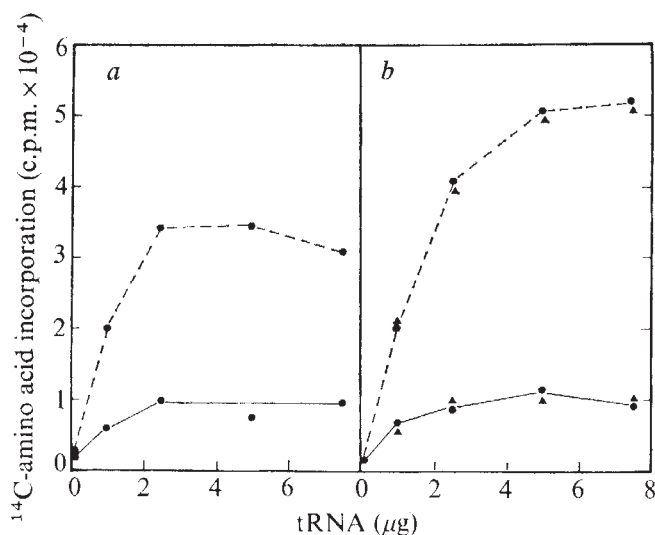
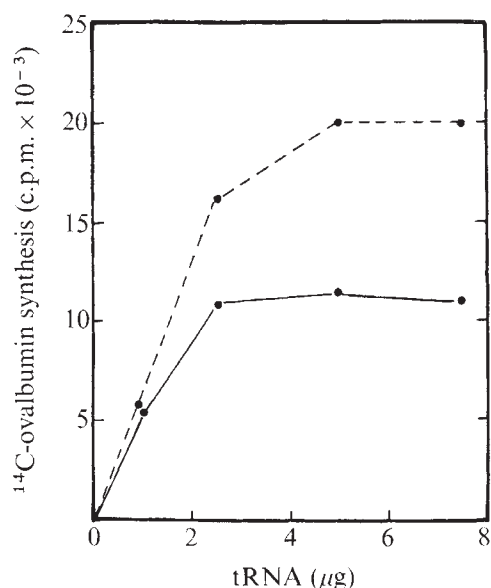


Fig. 1 Stimulation of protein synthesis by oviduct tRNA from immature chicks in a tRNA-dependent protein-synthesising system. The assay mixture contained in a volume of 100 μl: 20 mM Tris-HCl (pH 7.5), 1 mM ATP, 0.2 mM GTP, 6 mM creatine phosphate (all adjusted to pH 7.5), 20 μg creatine kinase, 0.5 μCi ¹⁴C-amino acid mixture, 0.05 mM of each of the six freshly neutralised non-radioactive amino acids missing from the ¹⁴C mixture, 6 mM mercaptoethanol, 50 mM KCl, 3 mM magnesium acetate, 3 μg oviduct mRNA, specified amounts of tRNA, 0.45 A₂₆₀ units of DEAE-cellulose-passed ribosomes and 0.12 A₂₈₀ units of ammonium sulphate fraction⁹. All incubations were carried out in duplicate at 37 °C for 90 min. The amino

acid incorporation was linear up to 120 min. At the end of incubation to determine amino acid incorporation into polypeptides, 0.2 ml 0.2 N KOH was added and the tubes were further incubated for 20 min, 1 ml TCA was added and the samples were filtered through Whatman GF/C filters, washed 4–5 times with 5% TCA and once with ethanol. The filters were dried and counted in a liquid scintillation counter. *a*, Stimulation of protein synthesis with oviduct tRNA from withdrawn chicks with endogenous mRNA (●—●) and amino acid incorporation in the presence of oviduct mRNA (●—●). *b*, Upper curve: stimulation of protein synthesis with oviduct mRNA by oviduct tRNA from oestrogen-stimulated chicks (●) or from laying hens (▲); lower curve: amino acid incorporation without oviduct mRNA. Four-day-old chicks were injected with oestradiol benzoate for 10 d followed by a withdrawal period of 3–4 weeks; the secondary oestrogen stimulation consisted of a single injection of 2 mg oestradiol¹¹. Oviducts were removed aseptically, the magnum portion of the oviduct was freed of adjoining tissue and was immediately frozen in acetone-dry ice. Frozen tissue was homogenised in 5 volumes 50 mM Tris-HCl, pH 8.3, 5 mM EDTA, 75 mM NaCl, 0.5% SDS and an equal volume of buffer-saturated phenol in a Sorvall omnimixer at full speed for 1 min. The aqueous phase was re-extracted twice with phenol and the nucleic acids were precipitated with 0.1 volume 20% potassium acetate and 2 volumes ethanol. The tRNA was separated from the bulk nucleic acids by DEAE-cellulose chromatography and was treated with DNase followed by Pronase⁸ and extracted with phenol. The tRNA was deacylated¹² and further purified by gel filtration on Sephadex G-100 (ref. 8). Oviduct magnum tissue from 40 chicks (4–5 g) withdrawn from oestrogen for 3–4 weeks and from 5–6 chicks (5–6 g) which received secondary oestrogen stimulation, was used for the isolation of tRNA. The yield of purified tRNA from withdrawn chick oviducts was one-half (10 A₂₆₀) of the tRNA from secondary oestrogen-stimulated chicks. Oviduct mRNA was isolated by affinity chromatography on oligo (dT)-cellulose¹³. All glassware was dried overnight at 300 °C and the solutions were made in glass-distilled autoclaved water.



globin mRNA no differences were found in the tRNAs from the two sources (data not shown). The quality of the tRNAs from the two sources in the translation of mRNAs other than that of ovalbumin is also implied, albeit to a quantitatively lesser degree, by the equivalent translation of the endogenous mRNAs from the ascites cells (Fig. 1, lower curves).

Oestrogens are known to have multiple effects on target tissues. In the chick oviduct they can stimulate accumulation of specific mRNA¹⁵⁻¹⁷, tRNA¹⁸, tRNA methyltransferases¹⁹, polysomes^{11,20}, endoplasmic reticulum^{14,21} and factors essential for translation²². The findings presented here imply that the relative inefficiency of oviduct tRNA from withdrawn chicks in ovalbumin synthesis may be due

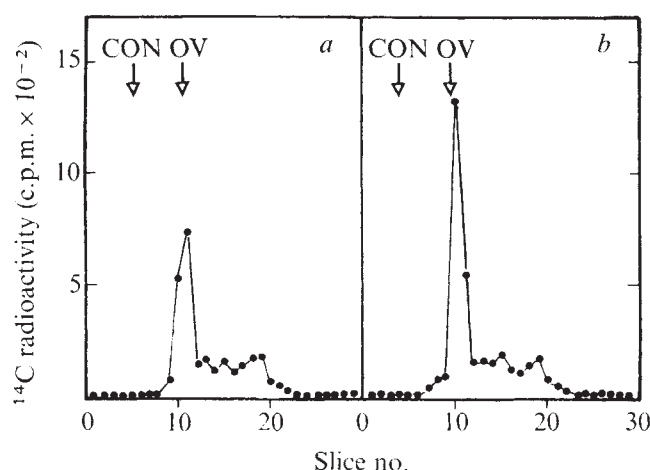


Fig. 3 SDS-polyacrylamide disc gel electrophoresis of labelled proteins precipitated with antiserum to ovalbumin. Details for protein synthesis and immunoprecipitation are described in Figs. 1 and 2. Immunoprecipitation was carried out in 25-μl aliquots of the reaction mixture of protein synthesis after 90 min incubation at 37 °C, washed antibody precipitates were mixed with ³H-labelled ovalbumin and conalbumin synthesised in oestrogen-stimulated oviduct explants¹⁴, as internal markers, and co-electrophoresed. The mobility of conalbumin (CON) and of ovalbumin (OV) is indicated. Oviduct mRNA was translated with 5 μg oviduct tRNA from withdrawn chicks (a) and oestrogen-stimulated chicks (b).

Fig. 2 Synthesis of ovalbumin in a tRNA-dependent protein-synthesising system. The details for protein synthesis were the same as described for Fig. 1. Ovalbumin synthesis was determined in aliquots of the assay mixture after incubation for 90 min at 37 °C by specific immunoprecipitation with antiserum against purified ovalbumin¹⁴. Aliquots (25 μl) of the reaction mixture were adjusted to 230 μl in plastic microfuge tubes (Beckman) by the addition of 15 mM NaCl in 10 mM sodium phosphate (pH 7.5); to this were added 10 μl ovalbumin (5 μg), 50 μl ovalbumin antiserum (antibody excess) and 20 μl 10% sodium deoxycholate. The contents of the tubes were mixed and incubated overnight at 37 °C. The precipitates were collected by centrifugation in Beckman microfuge (model 152), washed 3–4 times with 10 mM sodium phosphate (pH 7.5) containing 150 mM NaCl, 1% deoxycholate and 1% Triton X-100 (ref. 8). The pellet was dissolved in 0.1 ml NCS and counted¹⁴. Correction for nonspecific trapping of radioactivity by the immunoprecipitate was made by carrying out precipitation reactions in identical aliquots of the reaction mixture in the presence of 5 μg bovine serum albumin and 50 μl bovine albumin anti-serum. The immunoprecipitate from assay mixtures with oviduct mRNA usually contained 3,000–6,000 c.p.m. compared with 600–800 c.p.m. of nonspecific trapping. Ovalbumin synthesis was reproducibly quantified by this procedure. Ovalbumin-specific radioactivity was not precipitated from incubations in which rabbit globin mRNA or encephalomyocarditis viral RNA were translated. The specificity of the precipitation reaction was also checked by SDS-polyacrylamide gel electrophoresis of the antibody-antigen precipitate¹⁴. The radioactivity in the antibody-antigen precipitate from oestrogen-stimulated chick oviduct explants migrated as a single peak with the mobility of ovalbumin. Ovalbumin synthesis in the presence of oviduct tRNA from withdrawn chicks (●—●) and oestrogen-stimulated chicks (○—○).

to a deficiency in the population of tRNAs. The validity of such an assumption can be probed experimentally because the oviduct of the laying hen offers an abundant source of tRNAs which have a high efficiency of translation.

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Human-mouse cell hybrid with human multiple Y chromosomes

HUMAN-mouse cell hybrids usually exhibit preferential loss of human chromosomes^{1,2}. After a time interval, which depends in part on the cell types used, most or sometimes all the human chromosomes segregate out from the hybrid cells³. In the course of investigation of the phenomenon of

preferential loss of human chromosomes from hybrids between the mouse cell line RAG and human leukocytes, we isolated and analysed sixteen human mouse (RH) hybrids. Seven of these hybrids had retained the human Y chromosome. Five of these had only one copy per cell of Y, one had two copies per cell and one, RH-28, had four copies of the Y in many cells. We decided to study more extensively this hybrid which seemed to have lost all the human chromosomes except number 17 and the Y.

Figure 1 shows a cell of RH-28 stained by the Q-banding technique which has four human Ys and a human chromosome 17. We determined the distribution of Y chromosomes in 95 cells of RH-28. The results presented in Fig. 2 show that the majority of the cells screened had three or four Y chromosomes. In a few cells the number of Y chromosomes was even higher. In one cell which had a much larger number of mouse chromosomes there were ten human Y chromosomes. The only other human chromosomes present in these hybrid cells was a single copy per cell of chromosome 17.

The presence of multiple copies of a specific human chromosome in human-mouse cell hybrids has been reported by Green *et al.*⁴. In their hybrids, the mouse parent was a thymidine kinase deficient (*TK*⁻) mutant and Green *et al.* could, therefore, select for retention of human chromosome 17, which contains the *TK* gene. They tried to select lines with a high number of human chromosomes 17

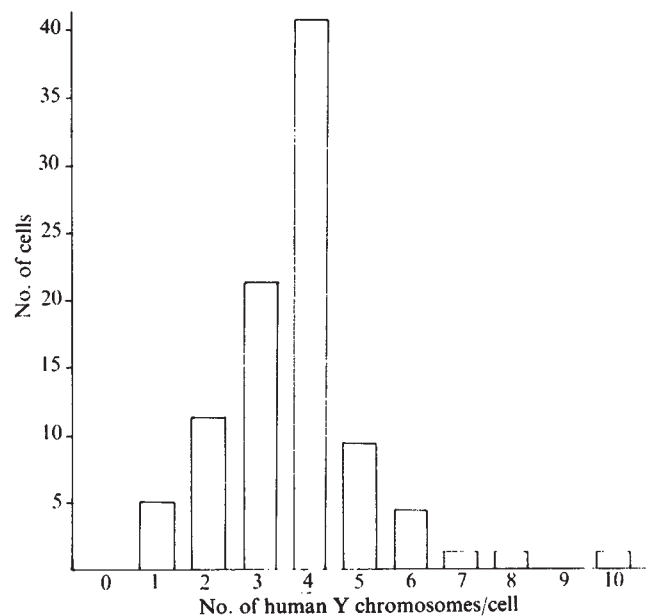


Fig. 2 Distribution of human Y chromosomes in cells of human-mouse hybrid RH-28.

by selecting subclones which grew in HAT medium containing low concentrations of thymidine. Three of ten subclones had higher mean numbers of chromosome 17.

We subcloned hybrid RH-28 to see whether we could select subclones in which all or most of the cells had the same number of Y chromosomes. The results (Fig. 3) indicate that in some of the subclones there was a stable number of Y chromosomes. Thus in subclone 8 all 25 cells scored had four Y chromosomes. In another subclone, 2, 30 of 34 cells scored had four Y chromosomes. In several other subclones the Y chromosomes seemed to have stabilised at a lower number per cell. In subclones 1, 3, 5

Fig. 3 Distribution of human Y chromosomes in cells from nine subclones of hybrid RH-28. The mean number per cell of human Y chromosomes and the mean number per cell of mouse chromosomes () is included for each subclone.

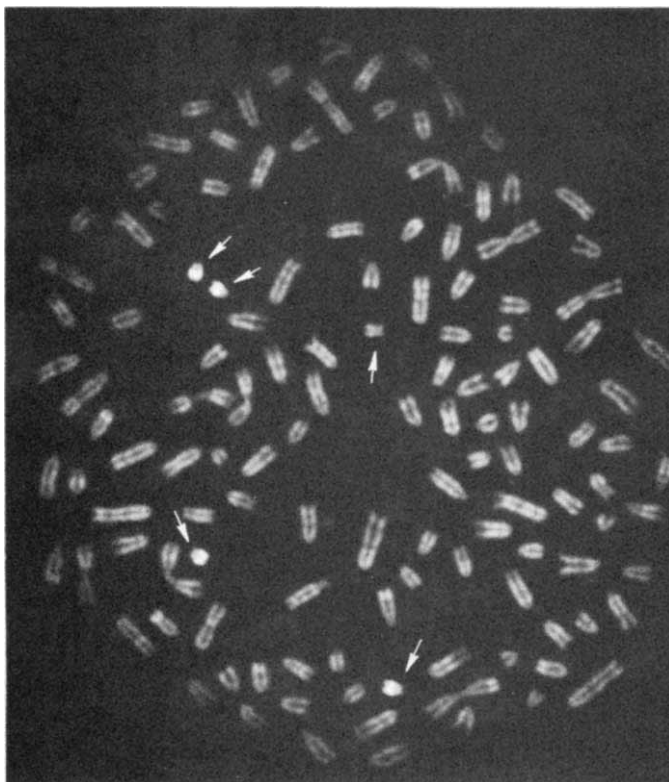
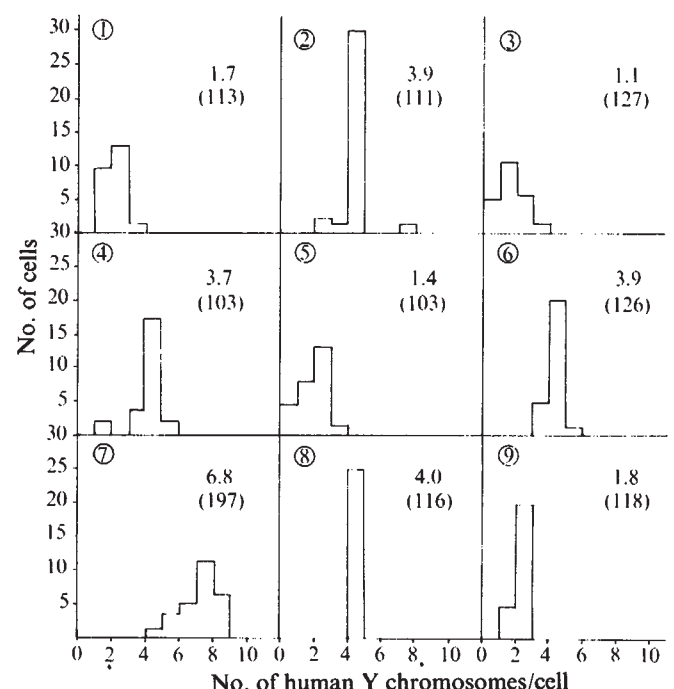


Fig. 1 A metaphase cell from RH-28 in which the human number 17 and four human Y chromosomes are indicated by arrows. The mouse parental line used in the hybridisation experiment was the hypoxanthine-guanine-phosphoribosyl-transferase deficient (HPRT⁻) RAG cell line⁸. The other parental cells were unstimulated leukocytes from a human male. The cells were induced to fuse by β -propio-lactone inactivated Sendai virus^{9,10}. Hybrids were selected in HAT medium¹¹. The isolated hybrids were maintained in MEM medium. Four-six weeks after fusion, cells from 16 hybrids were collected, chromosome preparations made and the chromosomes identified by quinacrine banding patterns¹².

and 9, 85 of 97 cells scored had only one or two Y chromosomes. A different situation was found in subclone 7 in which the number of mouse chromosomes doubled and the number of Y chromosomes in the majority of cells scored was between six and eight per cell. This subclone was stable over a period of at least 100 generations with respect to the doubled number of mouse chromosomes and the number of human Ys. In most of the cells of the nine subclones one copy of human chromosome 17 was still present.

If the accumulation of the Y chromosomes in hybrid RH-28 resulted from non-disjunction and selection for multiple Y chromosomes per cell, then the stability of some subclones with four and others with only one or two Y chromosomes indicates that the frequency of non-disjunction and selection has decreased. This phenomenon of accumulation of the Y chromosome may be analogous to the accumulation of B chromosomes in a variety of species⁵.

Mittwoch and Kirk⁶ showed that in mammals, gonadal cells which contain a Y chromosome grow faster than cells which do not contain a Y. It is tempting to speculate that the selection for cell hybrids with multiple Y chromosomes is due to a faster rate of growth.

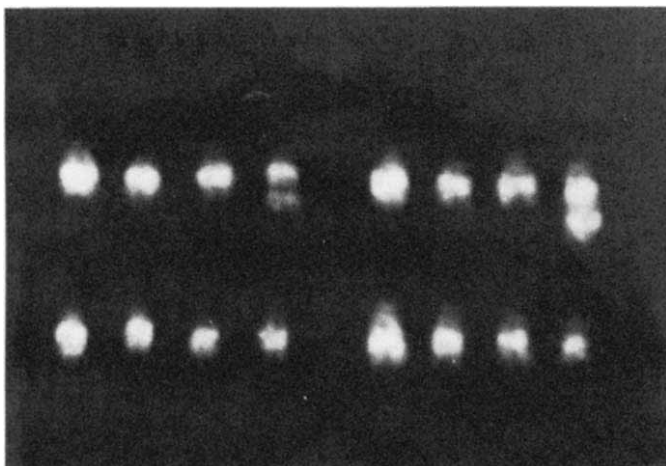


Fig. 4 Upper, human Y chromosomes from two cells of subclone 2. The Y chromosome on the right of each group is longer and has two quinacrine bright bands. Lower, human Y chromosomes from an RH-28 cell and a cell from subclone 6. Several of these Ys have a dull distal region.

In several subclones of the RH-28 hybrid the Y chromosome seemed to have undergone rearrangements which we are now investigating. Thus, in subclone 2 there were usually four Y chromosomes per cell. Three of these seemed to be normal whereas the fourth was almost twice as long and possessed two wide quinacrine bright bands instead of one (Fig. 4). In other cells, some of the Y chromosomes were altered in a different way, having a dull area distal to the bright band (Fig. 4).

The availability of human mouse hybrid cell lines containing the human Y may enable us to isolate and characterise proteins which are produced by Y linked genes, for example the H-Y antigen⁷. To facilitate this we have already obtained subclones that have lost the only other human chromosome present, number 17, but have retained multiple copies of the Y chromosome.

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Chromosomal proteins regulate steroid binding to chromatin

STEROID hormones accumulate in their respective target cells due to the presence of "receptor" proteins which bind the steroid with high affinity and specificity¹⁻⁴. It seems that the steroid-receptor complexes then migrate and bind to nuclear chromatin²⁻⁵. Within about 15 min there is an alteration in transcription which is followed after 2 h by the appearance of specific messenger RNAs⁶⁻⁹. At present, the mechanism by which steroids alter gene expression is obscure. The group in our laboratory is attempting to identify the nuclear (or chromatin) component which binds the progesterone-receptor (P-R) complex because it is possible that the macromolecules involved are also involved in the steroid-induced modifications of gene expression. We have demonstrated recently more than one class of binding sites in chick oviduct nuclei *in vivo* and in hen oviduct nuclei and chromatin *in vitro*^{10-12,13}. The highest affinity class of sites, representing 5,000-10,000 sites per cell nucleus, is suspected to be the biologically important class, because: (1) it is the only class which binds P-R *in vitro* in physiological ionic conditions (0.15-0.20 M KCl); (2) it is the only class bound *in vivo* when the steroid is at physiological levels in the serum; (3) binding to whole chromatin is tissue-specific, found only in oviduct chromatin, and (4) when these sites are saturated *in vivo* with the P-R, maximal responses of the RNA polymerase I and II activities are observed *in vitro*. Although additional nuclear binding to the sites of weaker affinity can be achieved *in vivo*, this causes no further alteration of polymerase activities¹³. We have also found that the low ionic conditions (0.01-0.05 M KCl), often used in binding assays, result in a pattern of nuclear binding which is non-saturable and shows no tissue specificity. When the ionic strength of the binding assays is increased to 0.15 M or 0.2 M KCl, there is a saturable, tissue-specific binding which measures selectively only the highest affinity class of binding sites¹⁰⁻¹². We now present evidence which suggests that one fraction of the non-histone protein (NHP) (called AP₃) is involved in the high affinity binding of P-R to the chromatin of the progesterone target tissue—oviduct—while other fractions (AP₁ or AP₂) are involved in masking these binding sites. We also present data which indicate that the high affinity binding sites are present but completely masked in non-target tissues.

Figure 1 shows high affinity binding (that is, binding assayed in 0.15-0.2 M KCl) of P-R to hen oviduct nuclei

and chromatin *in vitro*. The same high affinity binding to chick oviduct nuclei *in vivo* is shown for comparison. When the histones were removed, the *in vitro* binding was only slightly greater than that of whole chromatin. But when the acidic protein fractions AP₁ and AP₂ were also removed, the level of the high affinity binding to the oviduct material was enhanced four to fivefold. Finally, the removal of the remaining NHP (representing the AP₃ fraction) resulted in a loss of practically all high affinity binding. These results strongly support a role of NHP both in constituting part of the high affinity sites and in the masking of these sites in the oviduct (target tissue) nucleus. If the dissociated proteins were reconstituted back to the DNA, the high affinity sites were not only reinstated in the reconstituted hen oviduct chromatin but also at the same relative levels as the untreated hen oviduct chromatin, that is, the high affinity sites as well as the masking were restored. This ability to restore the native levels of binding to reconstituted chromatins was demonstrated previously with chick oviduct^{14,15}.

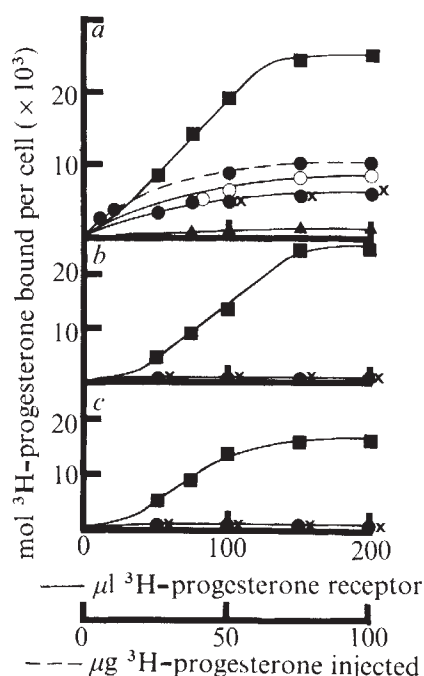


Fig. 1 Binding of ³H-progesterone to high affinity sites in the nuclear material of oviduct (a), spleen (b) and erythrocytes (c) of the chicken. — — —, Binding to nuclei of the chick oviduct *in vivo* as described elsewhere¹³; —, binding *in vitro* using isolated nuclear components and ³H-progesterone-receptor complex as described elsewhere^{11,12}. The *in vitro* binding to the soluble DNA-protein complexes was performed using streptomycin to precipitate the protein-DNA complexes, bound with the ³H-progesterone-receptor complex, or using protein-DNA complexes attached to cellulose as described elsewhere¹⁰. High affinity binding *in vitro* was assayed under 0.15 M KCl using 0–200 μl of labelled receptor and 50 μg of DNA (as chromatin or protein-DNA complex). The removal of total histones as well as AP₁, AP₂ or total protein has been described elsewhere^{2,12,14,15}. The efficiency of removing these fractions from chromatin DNA and the integrity of the nuclear components were assayed by polyacrylamide gel electrophoresis and quantitation of the protein: DNA using the Lowry¹⁸ and diphenylamine¹⁹ methods. The ³H-progesterone receptor complex was isolated, partially purified and assayed for its integrity as described elsewhere¹¹. The symbols represent binding to: ●, isolated nuclei; x, whole chromatin; ○, chromatin deficient in histone; ■, chromatin deficient in histone, AP₁, AP₂, and ▲, chromatin deficient in total protein (pure DNA). The number of molecules of ³H-progesterone bound per cell was calculated using the specific activity of the steroid (47 Ci mmol⁻¹ for the stock solution), 2.5 × 10⁻¹² g DNA per diploid cell of the hen organs²⁰ and 26% counting efficiency. The 10,000 molecules bound per cell represents 180,000 c.p.m. per mg of DNA or 9,000 c.p.m. per assay (50 μg DNA).

Table 1 Extent of masking of high affinity sites

Source	Level of binding at saturation (molecules per cell)	% Total sites masked
Oviduct nuclei <i>in vivo</i>	10,600	58
Oviduct nuclei <i>in vitro</i>	5,951	76
Oviduct chromatin	7,441	71
Oviduct chromatin -histone	9,278	53
Oviduct chromatin -histone -AP ₁ , AP ₂	25,290	0
DNA	2,055	—
Spleen nuclei	60	100
Spleen chromatin	48	100
Spleen chromatin -histone	—	—
Spleen chromatin -AP ₁ , AP ₂	24,106	0
Erythrocyte nuclei	357	98
Erythrocyte chromatin	416	97
Erythrocyte chromatin -histone	—	—
Erythrocyte chromatin -AP ₁ , AP ₂	15,018	0

These data were taken from values in Fig. 1. The number of binding sites measured in each of the chromatin-histone AP₁, AP₂ preparations was assumed to represent the total sites in the respective chromatin and thus each of these preparations was assigned a 0% masking. The values at saturation for each preparation were taken at 100 μg of injected hormone for the *in vivo* binding and at 200 μl of labelled receptor preparation for the *in vitro* binding. The preparations of the nuclear components as well as the hormone binding assays were performed as described in the legend of Fig. 1.

It is interesting that the nuclei and chromatin of non-target tissues (hen spleen and erythrocyte) showed little high affinity binding (Fig. 1). The removal of histones and NHP fractions AP₁ and AP₂, however, resulted in a level of binding approximating that of the target tissue (hen oviduct). Thus, although the non-target tissues showed none or a few nuclear high affinity sites, these sites were present but completely masked by protein (Table 1). Assuming that all acceptor sites were unmasked in the chromatins devoid of histones, AP₁ and AP₂, the *in vivo* binding to chick oviduct nuclei showed 58% masking. The *in vitro* binding to hen oviduct nuclei/chromatin exhibited about a 60 to 80% masking while that to hen spleen or erythrocyte nuclei/chromatin showed essentially 100% masking.

The biological need for an apparent distribution of high affinity binding sites throughout the animal and the masking of them remains obscure. It would be interesting to assess whether or not the extent of masking varies under normal biological influences. Along these lines, Chatkoff and Julian¹⁶ reported that progesterone treatment of ovariectomised rabbits caused an increase in the binding of oestrogen-receptor complex *in vitro* to uterine nuclei. Similarly, Spelsberg *et al.*¹⁷ found that the levels of binding of the progesterone-receptor complex to oviduct nuclei *in vitro* varied during the oestrogen-induced development of the chick oviduct. Both these studies involved the high affinity sites described here because physiological ionic conditions were used in binding assays. The question arises whether or not transformed cells in culture or in mammary tumours which contain steroid receptors but which do not respond to the steroids, have masked nuclear "acceptor" sites. Thus, these cells may be unresponsive since the genome cannot bind the steroid-receptor complexes.

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Effect of maternal age on intercellular adhesion of embryonic chick liver cells

In appropriate conditions single cells obtained by dissociation of embryonic tissues form organised cell masses resembling tissues. We wish to present results indicating that the adhesive properties of chick embryonic cells depend on maternal age. These unexpected observations were made in studies on the mechanism of intercellular adhesion. The formation of initial aggregates between chick embryonic liver cells is a two-step process¹; cells rapidly and reversibly bind to each other to form aggregates in a step that is not inhibited by metabolic poisons. The reversibly bound cells are then converted into stably bound cells in a process requiring metabolic energy.

Current studies are concerned with the formation of stable bonds between chick embryonic liver cells. The formation of such bonds was stimulated by the addition of L-ascorbic acid and L-glutamine (or D-glucosamine); these compounds promoted the adhesion of more single cells, and the resulting aggregates were larger and appeared more compact. As shown here, however, this stimulation depended on the age of the hen from which the embryo was derived.

The addition of ascorbate and glutamine to single cells derived from the embryos of younger hens (about 20 weeks old) increased the number of cells in aggregates after 40 min by 30-50% (Fig. 1). In contrast, the aggregation of cells derived from older hens (about 40 weeks) was not altered significantly by ascorbate and glutamine. The mechanism of action of ascorbate and glutamine is being studied and the results will be presented elsewhere.

Eggs from White Leghorn chickens (Truslow Farms, Maryland) and suspensions of liver cells from 12-d-old embryos were prepared by a modified method of Howard *et al.*². The cells, suspended in Earle's salt medium, were incubated on a rotary shaker (about 3×10^5 cells in 0.3 ml) and allowed to aggregate as described before¹. The rate at which single cells formed aggregates was determined with a Coulter electronic particle counter³. Only single cells were counted, so that the number of single cells remaining in suspension decreased as aggregation proceeded. The decrease in single cells was an accurate measure of cell aggregation.

In a typical experiment with embryos from younger hens

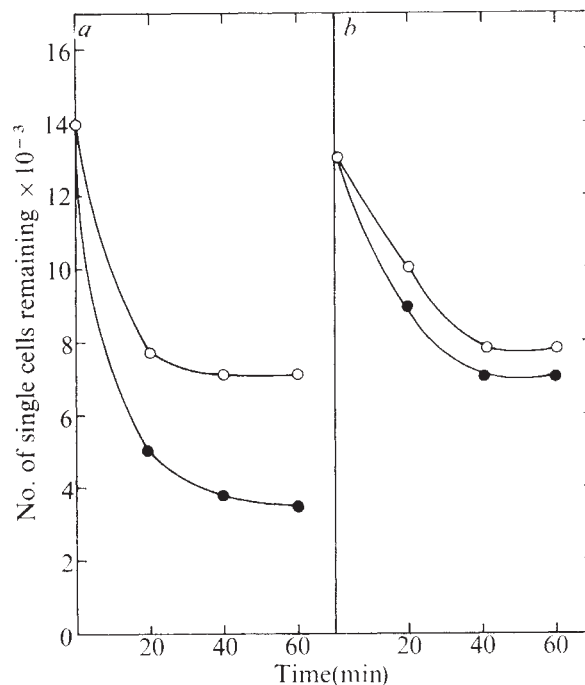


Fig. 1 Stimulation of liver cell aggregation by ascorbate and glutamine. Liver cells (2.9×10^5 cells, 0.3 ml) were allowed to aggregate at 37°C in a rotary shaker in standard conditions¹ in the presence or absence of ascorbate (0.6 mg ml⁻¹) and glutamine (2.7 mM). After incubation, cells were diluted with 10 ml of cold balanced salts solution and the single cells remaining (after dissociation of the reversibly bound cells) were counted. The data presented are not multiplied by the constant dilution factor. *a*, Liver cells of embryos derived from younger hens (27 weeks) were incubated without additions (○) or with ascorbate and glutamine (●). *b*, Liver cells of embryos derived from older hens (35 weeks) were incubated without additions (○) or with ascorbate and glutamine (●).

(27 weeks) 50% of the cells aggregated in 40 min. The addition of ascorbate and glutamine increased the extent of aggregation to about 75% (Fig. 1). When embryos from older hens (35 weeks) were used as a source of cells, however, no stimulation was obtained after addition of ascorbate and glutamine (Fig. 1).

The stimulation obtained by adding ascorbate and glutamine varied with the age of the laying hens (Fig. 2). This dependence on maternal age was observed with five different flocks of hens, and when 9- or 10-d-old embryos were used instead of 12-d-old hens. It was not influenced by the season of the year, indicating that the phenomenon was not affected by light-dark cycles nor moderate changes in temperature. The chickens were fed on an 18% protein breeder's mash without antibiotics, but with adequate vitamin C.

The livers of embryos from younger hens were also more readily dissociated into single cells than the livers of embryos from older hens. Not only was a higher yield of cells obtained after collagenase-hyaluronidase treatment of younger hens, but the preparations typically contained 50-80% single cells (the remaining cells were in aggregates of 2-20 cells) whereas livers from the embryos of older hens gave only 10-20% single cells.

Since the single cells were prepared with collagenase and it is well known that ascorbate is involved in the biosynthesis of collagen⁴, it seemed possible that both cell yield and response to ascorbate might be related to collagen structure. One aspect of the structure that is important for mechanical stability of collagen fibres is the amount of covalent intermolecular cross linking. Furthermore, the amount of collagen cross linkage is increased with age in many animals⁵. But treatment *in vivo* with β -aminopropionitrile

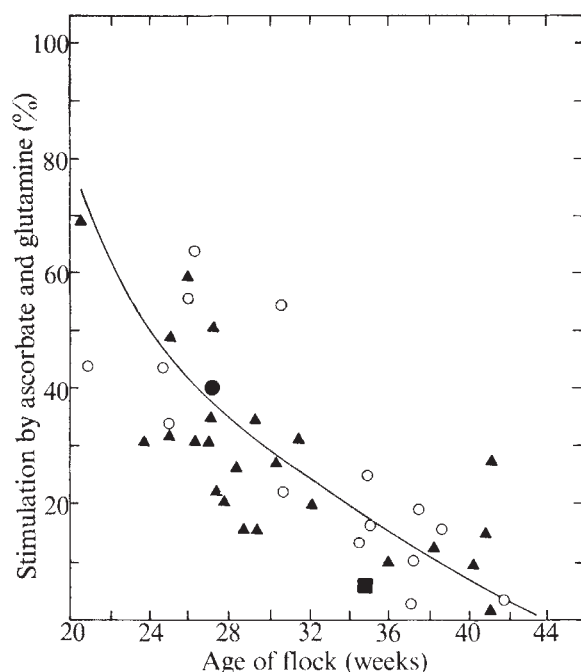


Fig. 2 Effect of maternal age on the stimulation of cell aggregation by ascorbate and glutamine. In each experiment about 3×10^5 liver cells were incubated in 0.3 ml of balanced salts solution on a rotary shaker at 37 °C. After 40 min, the samples were diluted with 10 ml of cold balanced salts solution and the number of single cells remaining was determined. To some of the vials L-ascorbate (0.7 mg ml^{-1}) and L-glutamine (27 mM) were added. The data presented here were obtained from two different flocks of chickens, one started in March (○) and one in August (▲). The abscissa indicated the age of the hens (note that efficient egg production began at 20 weeks), and the ordinate the percentage increase in the number of cells stably bound in aggregates caused by the addition of ascorbate and glutamine. The data in Fig. 1a were obtained in March and are indicated by ●; the data in Fig. 1b were obtained in May and are indicated by ■ in this figure.

(which inhibits cross linking^{6,7}) had no effect on the response to ascorbate *in vitro*, suggesting that collagen cross linking was not involved in the age-dependent stimulation of aggregation by ascorbate.

Although the mechanism underlying the observations reported here is unknown, maternal age seems significantly to affect the adhesive properties of embryonic cells *in vitro*. If *in vitro* adhesiveness as measured in these studies reflects *in vivo* histogenesis, the elucidation of the mechanism by which maternal age affects cell adhesion may ultimately shed light on the relationship between birth defects and maternal age.

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Limited proliferation of stem cells surviving alkylating agents

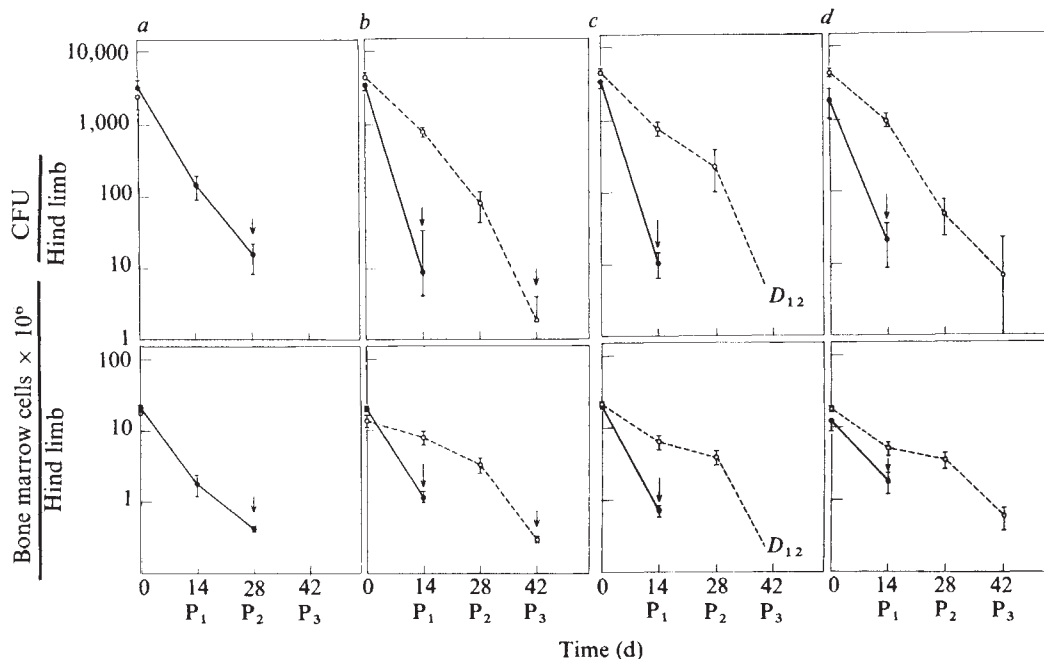
HAYFLICK observed that when human foetal tissue was cultivated *in vitro* the cells underwent 50 ± 10 population doublings before death¹. Whether this phenomenon is a property of all diploid cells in culture and is pertinent to cell renewal systems *in vivo* has been the subject of much discussion. Haematopoietic failure is rarely the cause of death in animal or man, presumably because this cell renewal system has a proliferative capacity which is either unlimited or, if limited, exceeds the life span of a normal animal. The proliferative capacity of the haematopoietic system, if restricted, might become exhausted when stressed by the prolonged use of cytotoxic agents. In these circumstances, significant stem cell division would be necessary to return to the steady state, thereby using much of the stem cell proliferative capacity. We have carried out experiments in mice attempting to simulate the clinical use of chemotherapeutic agents that might result in exhausting the proliferative capacity of haematopoietic stem cells. Two alkylating agents, Busulfan and L-phenylalanine (L-Pam) were investigated. Mice treated with Busulfan and to a lesser extent L-Pam demonstrate permanent damage to the proliferative capacity of surviving stem cells as measured by serial transplantation. Such data may have relevance to the use of these agents in the clinic where there is great interest in such agents to destroy presumed subclinical micrometastases in patients expected to have extended survival.

Using the haematopoietic system of mice, many investigators have attempted to demonstrate premature senescence *in vivo*. This system is useful because serial transplantation of bone marrow can be used as a measure of the proliferative capacity of haematopoietic cells. Further, the spleen colony-forming unit (CFU)² facilitates direct measurement of a stem cell population. Siminovitch *et al.*³ demonstrated a decline in colony-forming ability of haematopoietic cells subjected to serial transplantation into lethally irradiated recipient mice. Metcalf and Moore⁴ varied the interval between passages from 14 d to 187 d, and in spite of this increased interval observed progressive decline in CFUs per spleen. In a subsequent experiment, they used 10-d foetal liver and compared its transferability with that of marrow from both young and old mice. Foetal haematopoietic tissue sustained six passage generations, whereas young and old marrow cells survived only three such passages, suggesting that haematopoietic stem cells may have limited divisional capacity with most divisions taking place in the foetal period.

Others^{5,6} feel that limited proliferative capacity is not a characteristic of stem cells. With prolonged intervals between passage, the transplanted marrow tends to regain normal CFU levels. No method has been used, however, to distinguish host from donor CFU. When such determinations were made, it was found that with increased number of passages and long intertransplant time, donor cells disappeared and host cells predominated⁷.

We used C3H/HeJ male mice treated with Busulfan or L-Pam. The choice of Busulfan was based on the report by Morley and Blake⁸ showing that high doses of Busulfan can cause aplastic anaemia in surviving mice. The choice of L-Pam was based on the current clinical use of this drug for two years to treat presumed subclinical micrometastases in patients with seemingly localised breast cancer⁹. We suggest that certain drugs given in low doses might cause enough stem cell depletion and subsequent division to limit the proliferative potential of the haematopoietic stem cell. We used serial transfers as a method of increasing the number of stem cell divisions and thus elicit defects in the

Fig. 1 Comparison of transferability of normal (○) compared with Busulfan-treated marrow (●). Arrow denotes inability to passage further due to insufficient cells. D_{time} denotes date of death of last passaged mouse. *a*, 5; *b*, 10; *c*, 15; *d*, 20 weeks post-drug.



proliferative capacity of the stem cell population that might not otherwise become apparent.

To avoid drug-induced death, drug doses were chosen to correspond to half the LD_{50} (ref. 10) (0.18 mg Busulfan and 0.068 mg L-Pam). These agents were given intraperitoneally six times at weekly intervals. A control group was maintained in the same conditions as the experimental group and received weekly injections of the diluent. Peripheral blood counts were carried out weekly on tail vein blood samples of the experimental group during the period of drug administration and once every 2 weeks thereafter. No mouse was bled more than once every 16 weeks. Control mice were bled once every 4 weeks. Five weeks after drug completion, three mice from the control and experimental groups were killed for serial marrow passages into lethally irradiated recipients that received a whole body dose of 1,200 rad in divided doses of 700 and 500 rad separated by 3 h. The transfers were carried out every 5 weeks. One

million cells were injected into ten lethally irradiated mice at each passage interval, and 14 d separated each passage. At the time of each passage, marrow cellularity was determined, and ten lethally irradiated mice were injected for spleen colony determinations as described previously¹¹.

The effect of Busulfan on the ability to passage marrow cells is illustrated in Fig. 1. At 5 weeks after drug administration, the Busulfan marrow could be passaged for 28 d. At 10 weeks, passage was only possible for 14 d, as compared with 42 d for controls. These same patterns continue at 15 and 20 weeks post-drug. Marrow CFU per hindlimb before transfer was normal at 10 weeks, whereas at 15 and 20 weeks it had dropped to less than normal levels. On the other hand, marrow cellularity in the non-passaged state does not fall below normal until 20 weeks post-drug. Peripheral blood counts at all these transfer times (5–20 weeks post-drug) were normal.

The effect of L-Pam on the CFU is not as marked (Fig.

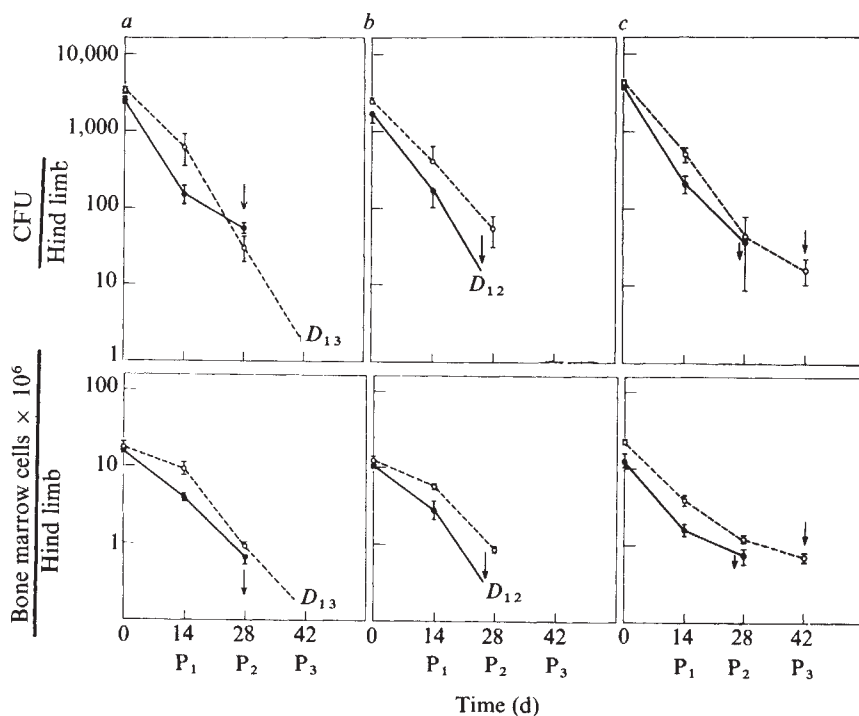


Fig. 2 Comparison of transferability of normal (○) compared with L-Pam-treated marrow (●). Arrow denotes inability to passage further due to insufficient cells. D_{time} denotes date of death of last passaged mouse. *a*, 5; *b*, 10; *c*, 15 weeks post-drug.

2). At all transfer times (5–15 weeks) L-Pam could not be passaged longer than 28 d, whereas control groups were kept in passage for 41 or 42 d. Marrow CFU and cellularity before transfer showed no difference compared with normal controls. The results are not as dramatic as those for Busulfan but are qualitatively similar as regards serial transplantation.

Considerations of seeding efficiency are complex and cannot easily explain the findings since with Busulfan the number of CFUs markedly decreased with each passage compared with control; whereas with L-Pam at 5 and 15 weeks the number of CFUs is similar to the control, but with both drugs there is a difference in serial transfer. There was no difference in spleen colony size between control and experimental groups at any stage of transfer. Detailed microscopic evaluation of the colonies, however, was not carried out.

Our results support the concept of there being limitations on the proliferative capacity of normal cells. Although limitation of proliferative capacity of the haematopoietic stem cell compartment is usually not an important constraint on life span, it may become critical when stressed by a prolonged application of, for example, Busulfan. An alternative but less likely explanation of these data is that the drugs cause the surviving cells to have a longer generation time and thus undergo less proliferation during each passage. This seems unlikely since the CFU number in the L-Pam groups after transfer was not decreased compared with control, although their ability to passage was decreased (28 compared with 42 d). Furthermore, Busulfan-treated mice showed a progressive decrease in passage ability (28 d at 5 weeks and 14 d thereafter), CFUs per hindlimb, and bone marrow cellularity per hindlimb.

Further work is necessary to determine whether marrow failure occurs as a result of these demonstrated defects in proliferative capacity. Although marrow failure may take many months to develop, its occurrence can be expected based on the results of Morley¹². Experiments using other classes of cytotoxic agents¹³ will be carried out to determine whether the stem cell can be spared by the use of such drugs. In the adult mouse and perhaps in man, the stem cells are proliferatively inactive; thus agents affecting only cells in cycle may be useful for destruction of micro-metastases without causing significant stem-cell depletion.

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Transformation of the β_2 adrenoceptor in normal rat liver into a β_1 type in Zajdela hepatoma

IN a large variety of tissues, malignant transformation is accompanied by changes in the cyclic AMP system¹: adenylate cyclase may be functionally absent or modified, while various quantitative alterations of hormonal membrane receptors may also occur². In liver, although the cyclase response to various activators may differ from one type of tumour to another³ as well as during the process of carcinogenesis itself^{4,5}, an enhanced response to catecholamines is generally observed^{4,6}. Here we describe gross modifications of the adenylate cyclase system in Zajdela ascites hepatoma cell line. Among these modifications is a hitherto undescribed qualitative alteration of the adrenoceptor.

Zajdela hepatoma, which was studied in the present work together with normal liver, was originally derived from parenchymal liver cells after chronic administration of dimethylaminoazobenzene⁷. It retains certain characteristics of the original cell⁷ and, for example, it can resynthesise glycogen in certain culture conditions⁸; thus it can be justifiably compared with normal liver^{9,10,11}. The animals used in this study were female, albino Wistar rats (~200 g body weight). A particulate fraction was prepared from normal liver by disruption with a Dounce homogeniser in 5 volumes (v/w) of 1 mM NaHCO₃. After two washing steps, which were performed by centrifugation at 2,000g for 10 min at 4 °C, the plasma membrane-enriched pellets were resuspended in the same NaHCO₃ solution (2 ml per g of liver). The corresponding fraction from Zajdela hepatoma was prepared from 20–50 ml of ascites fluid. Tumour cells were freed from contaminating erythrocytes by several washes in 0.2% NaCl. They were subsequently lysed in 1 mM NaHCO₃ for 10 min at 4 °C and the particulate fraction prepared as described for normal liver. As shown in Table 1, the cyclases from these two preparations had quite different activities in the absence as well as in the presence of stimulating agents. In the hepatoma, basal activity was ten times lower than in normal liver and hormonal responsiveness was altered in many ways. The effect of glucagon was detected only when its concentration was higher than 1 μ M, which corresponded to a supramaximal concentration in

Table 1 Adenylate cyclase activity of Zajdela hepatoma and normal liver

		Adenylate cyclase activity (pmol per mg protein per 10 min)	
		Normal liver	Zajdela hepatoma
Basal		95 ± 4	9.8 ± 0.5
+ Adrenaline	(50 μ M)	139 ± 6	170 ± 6
+ Glucagon	(1 μ M)	1,300 ± 30	17.1 ± 0.6
+ NaF	(10 mM)	1,010 ± 16	196.4 ± 4.5
+ GTP	(0.1 mM)	198 ± 25	9.2 ± 0.4
+ GTP + Adrenaline		340 ± 30	162 ± 5

Adenylate cyclase activity was measured as described previously¹². The assay medium contained 0.5 mM α -³²P-ATP (10^6 c.p.m.), 3 mM MgCl₂, 1 mM EDTA, 1 mM cyclic AMP, 50 mM Tris-HCl, pH 7.6, an ATP-regenerating system (consisting of 25 mM phosphocreatine and creatine phosphokinase (1 mg ml⁻¹) and the enzyme fraction (100–200 μ g protein per assay) in a final volume of 60 μ l. Incubation was initiated by the addition of the enzyme preparation and was performed for 10 min at 30 °C. Reactions were terminated according to White¹³. Protein was estimated by Lowry's procedure using bovine serum albumin as standard. Results are expressed in nmol of cyclic AMP formed in 10 min per mg of protein at 30 °C. Data represent the means $\pm$ s.e. of 3 experiments in which each point was assayed in triplicate. The mean responses differ from the basal activity with $P < 0.01$. It should be noted that the liver cyclase activated by catecholamines was completely inhibited by propranolol, a specific β antagonist. Addition of α -adrenergic blockers such as phentolamine or phenoxybenzamine did not alter the cyclase activity. α agonists such as oxymethazoline were completely ineffective (data not shown).

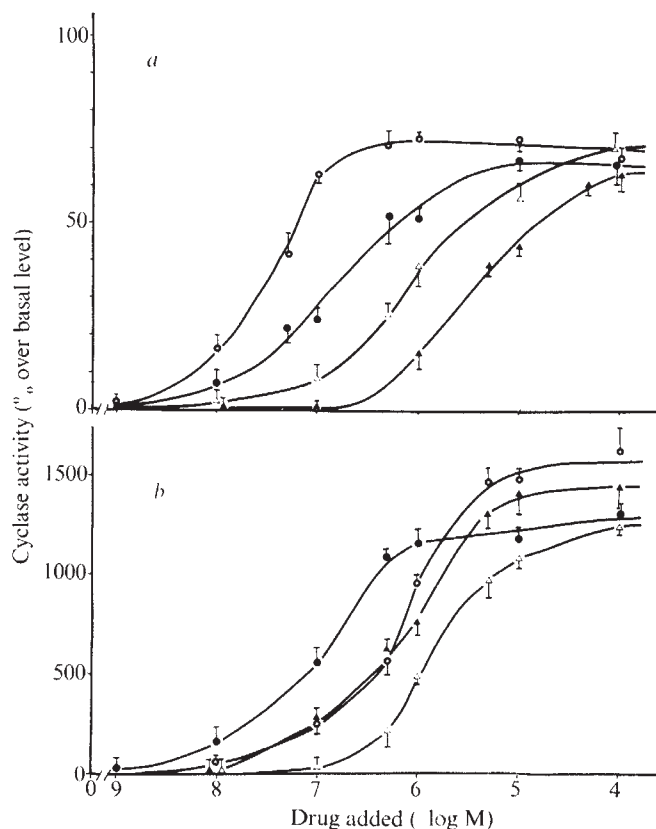


Fig. 1 Effect of various catecholamine agonists on cyclase activity. The enzyme preparations from normal liver (a) and from Zajdela hepatoma (b) were incubated as described in the legend to Table 1 and in the presence of varying concentrations of protokylol (○), isoproterenol (●), adrenaline (△) or noradrenaline (▲). The cyclase activity is expressed as percentage increase over the basal level (basal values are respectively 95 ± 4 and 9.8 ± 0.5 pmol cyclic AMP formed in 10 min per mg of protein in normal liver and in Zajdela hepatoma). Values represent the mean of three determinations; the standard errors are indicated by the bars. The apparent K_m s determined from this experiment and from two others are shown in Table 2.

normal liver¹⁴. In contrast, addition *in vitro* of $50 \mu\text{M}$ adrenaline effected a fifteenfold increase in the basal activity as compared to a twofold increase in normal liver. No synergistic effect of GTP could be observed in the present study (Table 1) although such an effect is a well known phenomenon in normal liver^{12,15}. Guanylyl imidodiphosphate, an analogue of GTP which is not a substrate for nucleotidases, was also ineffective, even at high concentration (0.1 mM). The relative fluoride response was, however, present in Zajdela hepatoma and even enhanced as compared to normal liver. These phenomena (that is, low basal activity and lack of effect of guanyl nucleotides), are probably indicative of marked changes in the catalytic component of the cyclase system. These changes are associated with a drastic alteration in the nature of glucagon binding or in its efficiency.

In view of the unusual character of cyclase stimulation by adrenaline in Zajdela hepatoma, its mechanism was further studied. The effects of various agonists were tested in a dose-response fashion and each experiment was repeated at least three times with different cyclase preparations from the normal liver and Zajdela hepatoma. In normal liver, the relative order of potency of the various agonists tested was the following: protokylol > isoproterenol > adrenaline > noradrenaline (Fig. 1a). This corresponds to a typical β_2 receptor^{16,17,18}; we obtained similar results with a purified plasma membranes preparation (unpublished data). It should be noted that protokylol (an analogue of isoproterenol characterised by a more hydrophobic substitution on the amino nitrogen), had an apparent K_m of 30 nM , and is the best β_2 agonist available in terms of affinity for the receptor. Similar results with this compound were reported previously by Rosen *et al.*¹⁹ for the β_2 receptor of frog erythrocytes. In Zajdela hepatoma cells (Fig. 1b), the order of relative potency of the various agonists was quite different: isoproterenol > protokylol > noradrenaline > adrenaline; while the affinity of isoproterenol was not modified, adrenaline became less potent than noradrenaline and protokylol less than isoproterenol. This corresponds to a β_1 receptor^{16,17}. The apparent K_m values for the various agonists were calculated in three different experiments and are listed in Table 2. The relative potency ratio (apparent K_m noradrenaline/apparent K_m adrenaline) was 4.8 for normal liver and 0.56 for Zajdela hepatoma. These ratios are similar to those reported by Burges and Blackburn for rat lung (β_2 receptor) and rat heart (β_1 receptor), namely 6.2 and 0.6 respectively¹⁷. In addition, we tested the effect of (–)-nordefrine, a relatively specific β_1 agonist¹⁶. Its apparent K_m was $5 \mu\text{M}$ in normal liver and $1 \mu\text{M}$ in Zajdela hepatoma. These values are similar to those found with noradrenaline ($4.3 \mu\text{M}$ and $1.3 \mu\text{M}$ respectively). To confirm this observation, further experiments were performed using specific antagonists. Their effect on adenylate cyclase was determined in the presence of a concentration of isoproterenol ($1 \mu\text{M}$) which resulted in a submaximal response. We used butoxamine as a β_2 inhibitor²⁰ and practolol as a β_1 inhibitor²¹. In normal liver, the isoproterenol-stimulated cyclase was blocked by 0.1 mM butoxamine, whereas practolol, at the same concentration, was completely ineffective. In contrast, in Zajdela hepatoma, $10 \mu\text{M}$ practolol inhibited cyclase activity by more than 75% whereas butoxamine was only slightly active at the same concentration.

It would seem, therefore, that in our system, malignant transformation not only provokes quantitative changes in the adenylate cyclase system, but is accompanied by a unique and hitherto undescribed qualitative change, namely the switch of the adrenoceptor from the normal β_2 type to the β_1 type. It should be noted that in the ascites hepatoma, the cyclase did not respond to unusual or inappropriate stimulating agents (for example, ACTH, dopamine, adenosine, serotonin). It is possible that the two different types of receptors coexist in liver and that the shift from a β_2 to a β_1 type corresponds to a change in the β_1/β_2 ratio. However, we favour the hypothesis that the catecholamine β

Table 2 Action of catecholamines on adenylate cyclase activity in normal liver and in Zajdela hepatoma

Agonist	Normal liver	Zajdela hepatoma
	Apparent K_m	
Protokylol	0.02 (0.03; 0.015; 0.015)	1.6 (2.5; 1.0; 1.3)
Isoproterenol	0.18 (0.20; 0.15; 0.20)	0.20 (0.37; 0.13; 0.17)
Adrenaline	0.9 (0.9; 1.0; 0.8)	2.3 (3.0; 1.6; 2.5)
Noradrenaline	4.3 (5.0; 5.0; 3.0)	1.3 (1.8; 1.0; 1.2)
	Relative potency	
Protokylol/isoproterenol	0.11	8
Noradrenaline/adrenaline	4.8	0.56

The apparent K_m is the concentration of agonist required to produce half maximal stimulation of adenylate cyclase. Results are the means of values from three experiments which were performed in a manner similar to that shown in Fig. 1; individual values are given in parentheses. Relative potency refers to the ratio of the apparent K_m of protokylol to that of isoproterenol, and of noradrenaline to that of adrenaline.

receptor constitutes a single entity susceptible to secondary, and possibly to reversible modification towards either type. Our results are the first demonstration of such a shift and seem consistent with recent data indicating a transformation between α and β receptors in certain physiological conditions^{22,23}.

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Effect of restriction endonucleases on infectivity of Rous sarcoma virus DNA

TRANSFECTION and molecular hybridisation assays show that animal cells infected with RNA tumour viruses carry DNA copies of the viral genome integrated into their chromosomes (for review see ref. 1). The transfection assay in particular enables the direct demonstration of the biological activity of the viral DNA^{2–4}. Shearing of the DNA⁵ and fractionation in sucrose gradients⁶ provided evidence that the virus is specified by DNA pieces of about 6×10^6 . Transfection kinetics⁵ and transfection probabilities at the endpoint dilution of the DNA^{1,7} show that one DNA piece of that size is sufficient to accomplish transfection.

Excision and extraction of the integrated viral DNA from cellular chromosomes requires an enzyme that will digest the cellular but not viral DNA. Such a selective digestion could be accomplished with restriction endonucleases for which there are no cleavage sites in the viral DNA. In this report we show that an enzyme from *Anabaena variabilis* (*Ava*I) satisfies these criteria, for it cleaves the DNA of cells transformed with Rous sarcoma virus (RSV) without abolishing the infectivity of this DNA.

DNA preparations from chicken cells transformed with RSV were used throughout the experiments because their high infectivity affords a reproducible transfection assay¹. One of these DNAs was extracted from a clone (No. 52)

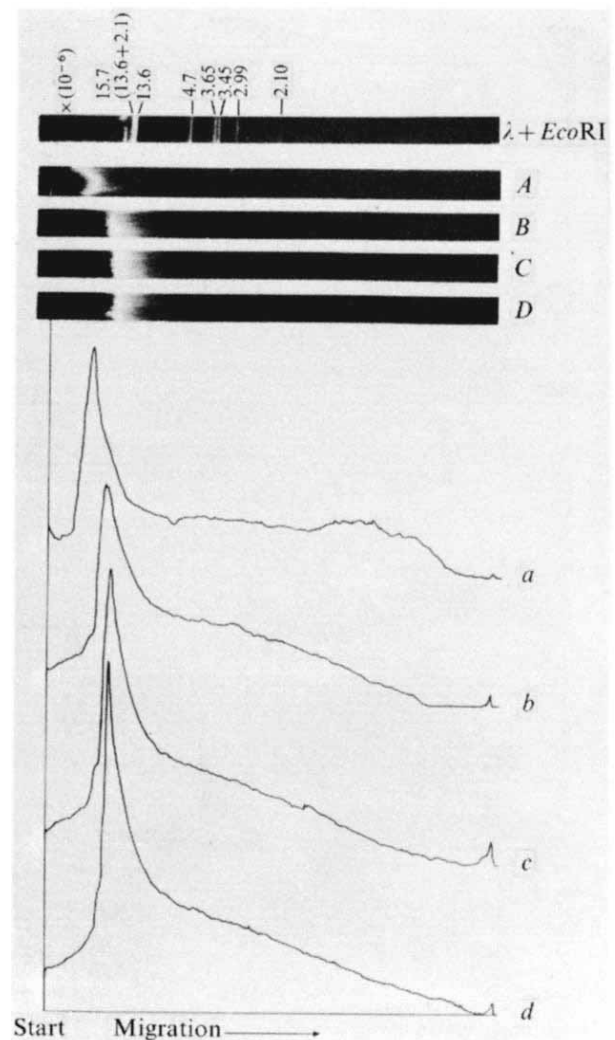


Fig. 1 Electrophoretic migration in 1% Agarose gels of phage λ DNA-*Eco*RI digest (λ +*Eco*RI), *nd,tdSR-D* DNA (A), and *nd,tdSR-D* DNA-*Ava*I digests (B, C, D). The *Eco*RI fragments of phage λ DNA were used as molecular weight markers. The *nd,tdSR-D* DNA was either mock-digested for 9 h (A), or digested with *Ava*I endonuclease for 3 h (B), 6 h (C), and 9 h (D). The Joyce-Loebl densitometry tracings of A, B, C, and D are shown in a, b, c, and d, respectively.

of cells derived (with all probability) from a single chicken embryo fibroblast infected and transformed with a single particle of the Schmidt-Ruppin RSV, subgroup D (SR-D). The origin of the virus and the cells, the cloning procedure and the extraction of the DNA have been described elsewhere⁸. In transfection assays, virtually all of the chicken cell cultures which received $5 \mu\text{g}$ of this DNA gave rise to a non-defective (*nd*) sarcoma virus (ref. 1 and J.H., and M.H., submitted for publication). Thus the DNA is named *ndSR-D* DNA. The other DNA used was extracted from mass cultures of chicken fibroblasts transformed with the same SR-D virus as before. In contrast to the first DNA, this preparation gave rise, following transfection, to both *nd* and transformation-defective (*td*) viruses⁸ and therefore is called *nd,tdSR-D* DNA. The dose-response relationships in transfection assays with these two DNA preparations have been described elsewhere¹, where it was also shown that *td* viruses arise uniquely from *tdSR-D* DNA.

The restriction endonucleases used in this study are listed in Table 1. Each enzyme was assayed by incubation with $1.5 \mu\text{g}$ of phage λ or SV40 DNA at 37°C . Incubation times and references for the incubation conditions with different enzymes are given in Table 1. The DNA digests

Table 1 Transfection assays with undigested and restriction enzyme digested SR-D DNAs

Experiment	DNA	Restriction endonuclease, time of incubation (h) (ref.)		Transfection efficiency
1	<i>nd</i> SR-D DNA	none	16	3 <i>nd</i> /3*
		<i>Hind</i> II,	16 (14, 15)	0/3
		<i>Hind</i> III,	16 (15, 16)	0/3
		<i>Hind</i> III + III,	16 (17)	0/3
		<i>Hae</i> III,	16 (18, 19)	0/3
		<i>Hpa</i> I,	16 (9)	0/3
		<i>Hpa</i> II,	16 (9)	0/3
		<i>Eco</i> RI,	2.5 (20)	0/3
2	<i>nd,td</i> SR-D DNA	none,	2.5	3 <i>nd</i> /3
3	<i>nd</i> SR-D DNA	none,	2.5	2 <i>nd</i> /3
		<i>Ava</i> I,	2.5†	1 <i>nd</i> /3
		<i>Ava</i> II,	2.5†	0/3
4	<i>nd</i> SR-D DNA	<i>Ava</i> I,	3†	1 <i>nd</i> /3
5	<i>nd</i> SR-D DNA	<i>Ava</i> I,	3†	2 <i>nd</i> /3
	<i>nd,td</i> SR-D DNA	none,	3†	3 <i>nd</i> /3
6	<i>nd,td</i> SR-D DNA	<i>Ava</i> I,	3†	2 <i>nd</i> + 1 <i>td</i> /3
		none,	9†	3 <i>nd</i> /3
		<i>Ava</i> I,	3†	2 <i>nd</i> /3
		<i>Ava</i> I,	6†	1 <i>nd</i> /3
		<i>Ava</i> I,	9†	1 <i>nd</i> + 1 <i>td</i> /3

Transfection assays were carried out by means of DEAE-dextran (experiment 1) or calcium phosphate (experiments 2 to 6) in conditions described in refs 8 and 10. Each culture received 5 µg of undigested or digested DNA. The DNAs were extracted from SR-D-transformed chicken cells and consequently contained both the cellular and viral DNA as described in the text. *Eco*RI and *Ava* enzymes were provided by Drs A. Bernardi and G. Roizès, respectively. The *Haemophilus* enzymes were purified according to the procedure described in ref. 21. Incubation conditions of the DNA with the respective restriction enzymes are given by references in the table.

*Number of transfected (that is, virus producing) cultures per number of DNA-treated cultures. The transfected cultures are designed with *nd* or *td* to indicate that the DNA gave rise to a transforming or non-transforming virus, respectively.

†K. Murray, S. G. Hughes, J. S. Brown, and S. Bruce, submitted for publication.

were analysed by electrophoresis in 1% Agarose gels and the resulting bands were located under ultraviolet light after staining with ethidium bromide⁹.

The DNA (15 µg) extracted from chicken cells transformed with SR-D was mixed with appropriate amounts of the restriction enzymes and incubated in the conditions used for the enzyme assays (see also Table 1), and the reactions stopped by addition of 0.1 volume of 0.1 M EDTA. Then the volume of the mixture was adjusted with 0.14 M NaCl-0.05 M Tris-HCl, pH 7.4, or HEPES-buffered saline solution (NaCl, 8.0 g l⁻¹; KCl, 0.37 g l⁻¹; Na₂HPO₄·2H₂O, 0.125 g l⁻¹; dextrose, 0.1 g l⁻¹; N-2-hydroxyethylpiperazine-N-2-ethane sulphonate, 5.0 g l⁻¹, pH 7.05) to make 6 ml, and the mixture was distributed into three secondary chicken cell cultures to assay its infectivity by means of the DEAE-dextran⁸ or calcium phosphate¹⁰ techniques, respectively. The cultures were kept growing for about 3 weeks (through four to five passages) and examined for the occurrence of transformed cells and, in non-transformed cultures, by reverse transcriptase assay¹¹ for type C particles in the culture medium.

The results are summarised in Table 1. Of the eight restriction endonucleases, seven rendered the DNA non-infectious. This suggests that RSV DNA contains cleavage sites for the enzymes *Hind*II, *Hind*III, *Hae*III, *Hpa*I, *Hpa*II, *Eco*RI, and *Ava*II. The alternative view that loss of infectivity results from the cleavage of cellular rather than viral DNA sequences is improbable because mechanical shearing of the DNA preparations down to the average size of 6 × 10⁶ daltons does not abolish their infectivity³⁻⁷ and unintegrated viral DNA obtained from mouse cells early after infection with a murine leukaemia virus is infectious¹² though this DNA presumably lacks cellular sequences.

The case of the eighth enzyme, *Ava*I, was different. The electrophoretic analysis (Fig. 1) showed that digestion with *Ava*I gave a continuous distribution of fragments in which no sharp bands were visible, but with a maximum abundance of fragments larger than 20 × 10⁶ daltons (as determined from comparison with migration patterns of phage λ

DNA-*Eco*RI digest¹³). It is worth adding that the migration pattern of the 3-h digest was essentially the same as those of 6-h and 9-h digests (Fig. 1). This indicates that virtually all the *Ava*I sites of the DNA were cleaved within 3 h of incubation. In transfection assays (Table 1) the DNA cleaved with *Ava*I repeatedly gave rise to the virus though with a somewhat lower efficiency than that obtained with undigested DNA. *A priori* such a difference in transfection efficiency could be attributed to a diminished uptake by recipient cells of DNA molecules of smaller size rather than to the cleavage of certain, but not all, molecules of viral DNA by the *Ava*I restriction enzyme. The possibility of incomplete digestion of the DNA by the enzyme was eliminated in experiment 6 (Table 1) showing that the DNA infectivity resists much longer incubation times than that (3 h) sufficient for exhaustive cleavage (Fig. 1).

Table 1 also shows that both *nd* and *td* viruses were recovered following transfection with *nd,td*SR-D DNA that had been treated with *Ava*I. This hardly surprising observation implies that no *Ava*I cleavage site is formed when a *td* virus DNA is generated from RNA (or less likely (ref. 1 and J.H., and M.H., submitted for publication) DNA) of the non-defective parent.

In conclusion the transfection assays show that cleavage sites for *Hind*II, *Hind*III, *Hae*III, *Hpa*I, *Hpa*II, *Eco*RI, and *Ava*II restriction endonucleases occur within SR-D DNA. In contrast, both *nd* and *td* SR-D DNAs lack cleavage sites for *Ava*I endonuclease. This offers the means for isolation of fragments of cellular DNA containing the integrated viral DNA and the analysis of cellular DNA sequences adjacent to the integrated viral genome.

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Spontaneous transformation of human skin fibroblasts derived from neoplastic patients

In the past 15 years a considerable amount of data has been published showing that normal human fibroblasts have a finite division potential when cultured *in vitro*^{1,2}. We have described the spontaneous establishment *in vitro* of stromal fibroblasts derived from human lung cancers³, but it was difficult to know whether the proliferating fibroblasts were normal stromal cells or tumour cells. Therefore we decided to culture the fibroblasts from the skin of patients with lung tumours to see whether they had the same fate *in vitro*. Here we describe the spontaneous transformation of skin fibroblasts derived from four neoplastic patients.

Skin biopsies from four cancer patients were obtained from the edge of the marginal wounds at the beginning of

the operation. Three of these patients had lung cancer and one had a solitary plasmacytoma of the mediastinum. At the time of the biopsy the patients had not been on chemotherapy. Tissue samples were cut into small pieces with fine forceps and four to five fragments were placed into 30-ml plastic flasks. Each fragment was covered with a drop of medium and incubated in 90% air:10% CO₂ at 37 °C. After 24 h, 5 ml of medium was added to each flask. When the fibroblast outgrowth covered the surface of the culture vessels, the cells were detached with a trypsin-versene solution and transferred into two new flasks (1:2 split). Cultures were then subcultured routinely with 1:2 splits when the cells were confluent. The medium used throughout was Eagle's MEM supplemented with non-essential amino acids, 15% foetal calf serum, penicillin (100 U ml⁻¹) and streptomycin (100 µg ml⁻¹). Control cultures from thoracic skin biopsies of sex-matched but not age-matched normal donors were initiated and continued in the same conditions.

During the first five subcultures the cells from the cancer patients looked like normal fibroblasts disposed in orderly arrays. Between the sixth and the ninth passage, foci of epithelial-like cells began to appear. The latter progressively invaded the cultures, forming a criss-cross pattern with multilayering and random orientation. Cytogenetic analysis, performed at different passages (summarised in Table 1) showed that in all cultures derived from the skin of patients with lung tumours, an abnormal chromosome distribution appeared during the early phases of culture, contrasting with the near normal distribution found in the control skin fibroblasts and in WI-38 cells⁴.

The colony-forming capacity in a semi-solid agar medium was also checked⁵. Fibroblasts from normal donors did not form colonies. The cells from the patients, however, gave rise to more than 0.5% colonies. The cultures derived from donors with lung tumours were also found to form aggregates in the presence of concanavalin A⁶ whereas the control cultures did not. From each culture derived from the patients it was possible to obtain cells which overcame phase III (ref. 1) and became established.

All cultures derived from normal donors went through the normal evolution previously reported^{1,2} and died after serial subculture. Finally we should emphasise that when the fibroblasts were derived from skin explants and adapted to *in vitro* culture, and when transformation occurred, our laboratory contained no HeLa cells or other cell lines, which could have been sources of contamination.

Our results show that it is possible to obtain spontaneous transformation in cultures obtained from the skin biopsies

Table 1 Number of mitoses with different sets of chromosomes found in human fibroblasts cultivated from the skin of normal donors and from patients with lung tumours

Donor	Cell culture	Passage* mitoses scored	No. of chromosomes											
			< 45	45	46	46-50	50/60	60-70	70-80	80-90	90-92	92-100	> 100	> 200
Normal Donors	SK-TOR	XIV/50		1	49									
	SK.1	IV/45			45									
	SK. 2	XII/50			50									
	WI 38	XVI/46			38									
Patients	Falla	I/50	18	14	16						2			
		†VII/50			1		27	5	1	2	2		12	1
	Nico	I/24	5	1	15						2		1	
		II/33	3	2	20		2				4		2	
		III/21	2	5	12		1				1			
		†X/50	4		3	3	31	6			1		1	1
	TAM	VI/50					16	23					11	
		†VI/50			1		9	24		1	1		12	2
	Ziani	Primary/30	5	4	16		2				2		1	
		I/37	8	4	11		7				4		3	
		II/50	14	3	25		2				6			
		†XII/50			1		40	7			1		1	

*Passages at which cytogenetic analysis was made. A standard procedure using Colcemid and acetic acid orcein staining was used.

†Number of passages after transformation has occurred.

of patients with lung tumours. Transformation is characterised by an abnormal ploidy, a capacity to form colonies in a semi-solid agar medium, the formation of foci of piling up cells among the monolayers and the acquisition of an infinite life span. For the moment it is impossible to know if the transformed cells originated from skin fibroblasts or from tumour cells disseminated in the skin of the patients.

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Enumeration of specific cytotoxic T cells

EFFORTS directed towards the enumeration of effector cells involved in the cell-mediated immune response have so far met with little success. One of the principal reasons for this failure is the current ignorance of relationships between killer, lymphokine producer and suppressor T cells, all of which have effector functions. To date, attempts at quantification have focused principally on cytolytically active (killer) T cells largely because *in vitro* assays using target cells labelled with radioisotopes afford sensitive and reproducible measurement of their function¹.

Two factors have, however, hampered the enumeration of the killer cells in cytolytically-active lymphoid cell populations: (1) the effector T cell can kill more than one target cell^{2,3} and (2) the number of effector cells cannot be distinguished from their intrinsic lytic efficiency. The latter factor is presumably a complex function comprising the mobility of effector cells, their avidity for target cells and their ability to lyse and disengage from targets. Standard assay conditions for *in vitro* cytotoxicity enable comparisons to be made between the lytic activities of different "immune" lymphoid cell populations, but these comparisons may bear little relation to the relative numbers of effector cells if there is wide variation in killing efficiency. We describe here an experimental approach in which efficiency is maximised and the number of cytolytically-active T cells within a lymphocyte population is determined by preventing killer cells from lysing more than one target.

The method was based on two findings: (1) Lymphocyte-target cell interactions take place in the presence of Mg²⁺,

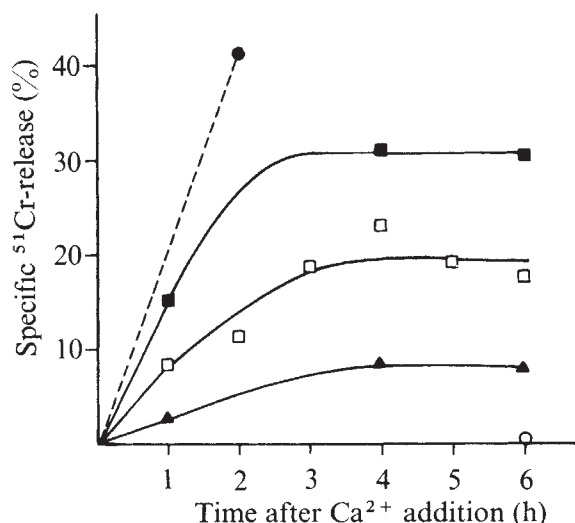


Fig. 1 Spleen and ⁵¹Cr-labelled tumour cells were washed twice in Ca²⁺-free medium (MEM-suspension, Microbiological Ass., containing 5 × 10⁻⁵ M EDTA to remove trace amounts of Ca²⁺, and 5% dialysed foetal calf serum) just before use. Spleen (50 µl) and target cells (50 µl, 10 × 10³) were added to conical-well microtitre plates which were centrifuged for 1 min (1,000 r.p.m. No. 276 International Centrifuge rotor, 22 °C) and incubated for 1 h at 37 °C. Cytochalasin A (50 µl, 30 µg ml⁻¹, Aldrich; the stock solution, 10 mg ml⁻¹ in dimethyl sulphoxide, was diluted with Ca²⁺-free medium before use) was then added, followed 10 min later by 100 µl Ca²⁺-containing medium (MEM-Earle's Salts, Microbiological Ass., containing 5% foetal calf serum). The specific release was calculated as follows:

$$\text{Specific release} = \frac{\text{Experimental c.p.m.} - \text{control c.p.m.}}{(0.8) \times (\text{incorporated c.p.m.})}$$

where 0.8 is the fraction of the incorporated ⁵¹Cr which is releasable by hypotonic lysis. The controls were spleen cells from uninjected C57BL/6 mice treated identically to the "immune" cells. The control release was 5% per h for all conditions. The following numbers of spleen cells were used: ▲, 2.5 × 10⁵; □, 5 × 10⁵; ■, 10 × 10⁵; ●, 5 × 10⁵ (no cytochalasin A was added); ○, 5 × 10⁵ (no Ca²⁺-containing medium was added).

but in the absence of Ca²⁺ these lesions do not lead to cytotoxicity⁴. (2) Cytochalasin A inhibits cytotoxicity (irreversibly) and does so by interfering with a stage in the lytic cycle which precedes the Ca²⁺-dependent event(s). (It seems likely that cytochalasin A inhibits lymphocyte-target cell interactions, as does its congener cytochalasin B (ref. 5), although the validity of this assumption is unnecessary for the arguments which follow.)

Thus, by incubating lymphocytes with target cells in a medium containing Mg²⁺ but not Ca²⁺, interactions occur which do not lead to lysis. If Ca²⁺ is added to such cultures together with cytochalasin A, those target cells which have functionally engaged with effector cells during the pre-incubation period will go on to lyse. The presence of cytochalasin A will, however, prevent any new interactions from proceeding to a Ca²⁺-dependent stage, and thus new liaisons formed after the addition of Ca²⁺ will not lead to lysis. Assuming each effector cell becomes bound to one target cell during the preincubation period, the subsequent number of target cells which lyse when Ca²⁺ is added to the cultures is equivalent to the number of effector cells present.

Cytolytically active lymphoid cell populations were generated by immunising adult C57BL/6 mice (Jackson Laboratories) with a mouse mastocytoma (P815-X2) of the DBA/2 strain. Ten million P815 cells were given intraperitoneally and the lymphoid cell populations collected 10-12 d later in a manner previously described in detail⁶. The cell-mediated destruction of P815 cells in this system has been shown to be exclusively due to T cells⁷.

Specific cytotoxicity was totally inhibited when lymphocytes

Table 1 Maximising the interaction between effector and target cells

Target cells added (× 10 ⁻³)	Target cells killed (× 10 ⁻³)	
	3 h	5 h
25	2.8 ± 0.1	2.8 ± 0.2
50	3.6 ± 0.3	3.8 ± 0.3
100	3.7 ± 0.5	3.6 ± 0.4
200	3.6 ± 0.7	4.0 ± 0.5

Spleen cells (5 × 10⁵) from sensitised animals were incubated with the indicated number of ⁵¹Cr-labelled P815 target cells as described in Fig. 1. After 1 h at 37 °C, cytochalasin A was added and 10 min thereafter Ca²⁺-containing medium. The number of target cells specifically lysed (± s.e.m. for duplicates) was determined 3 and 5 h after the addition of Ca²⁺-containing medium to show that maximum lysis had occurred.

Table 2 Specific cytolysis as a function of interaction time in Ca^{2+} -free medium

Ca^{2+} -free incubation time before cytochalasin addition (minutes)	% Specific Lysis	
	3 h	5 h
0	0.0 ± 0.1	0.0 ± 0.1
30	20.5 ± 0.1	21.0 ± 0.2
60	23.0 ± 0.2	23.5 ± 0.1
120	22.5 ± 0.1	25.0 ± 0.1

Spleen cells (1×10^6) from sensitised animals and ^{51}Cr -labelled P815 target cells (2×10^4) were mixed in conical-well microtitre plates and centrifuged as described in Fig. 1. After the incubation times indicated cytochalasin A was added and 10 min thereafter Ca^{2+} -containing medium. The percentage-specific lysis ($\pm$ s.e.m. for duplicates) was determined 3 and 5 h after the addition of Ca^{2+} -containing medium.

and target cells were incubated in the presence of $3 \mu\text{g ml}^{-1}$ (6×10^{-6} M) cytochalasin A. Lymphoid cells preincubated for 10 min with cytochalasin A and then washed did not recover their lytic activity, but target cells treated in an identical manner were as susceptible to lysis as untreated cells (data not shown). The drug did not increase the rate of spontaneous release of ^{51}Cr from target cells (5, confirmed in experiments not shown).

The experimental protocol used to enumerate effector T cells is given in detail in Fig. 1. (Cytochalasin A was added 10 min before Ca^{2+} to allow a finite time for the drug to exert its effect.) No cytolysis occurred when lymphocytes were incubated with target cells in a medium containing Mg^{2+} but not Ca^{2+} (Fig. 1). On the addition of Ca^{2+} , however, ^{51}Cr release readily occurred (Fig. 1). Two features of the target cell destruction following the addition of Ca^{2+} should be noted. (1) The number of target cells killed was proportional to the number of spleen cells present in the culture; (2) a period of about 3 h in medium containing Ca^{2+} was necessary to allow maximal ^{51}Cr release. To assure that the drug was effective and that the maximal ^{51}Cr release had occurred, lysis was determined 3 and 5 h after the addition of Ca^{2+} in the presence and absence of cytochalasin A.

If, as is our contention, the maximal amount of target cell destruction depicted in Fig. 1 is equivalent to the number of effector cells present in the lymphocyte population, several provisions must hold. First, for a given effector cell population, the maximum specific target cell destruction after the addition of Ca^{2+} to the cultures, should be directly proportional to the number of lymphocytes present. The data in Fig. 1 show this to be the case. Second, increasing the size of the target cell pool, above the minimum required to engage all effector cells, should not further

increase the number of target cells killed. When 5×10^5 immune spleen cells were incubated with between 5×10^4 and 20×10^4 target cells, the number of target cells killed after the addition of Ca^{2+} was constant (Table 1). Third, the number of target cells killed should not be increased if the incubation period in Ca^{2+} -free medium is extended (that is, a maximal number of interactions had occurred in the preincubation time chosen). The number of functional engagements between lymphocyte and target cell did not significantly increase over the period 30 min to 2 h (Table 2), in keeping with previous studies^{2,8}.

Since these criteria were fulfilled, the number of target cells maximally killed was equated with the number of effector cells. This approach was used on several occasions to enumerate effector cells in C57BL/6 spleen cell populations at the peak of the primary immune response (10–12 d) after immunisation with P815 cells. Table 3 shows that at this time between 0.7 and 2.2% of the total spleen cell population was specific anti-P815 T effector cells.

There are two principal assumptions necessary in order for our calculations to be valid. First, all effector cells must functionally interact with a target cell during the preincubation period (obviously if some effector cells do not engage a target cell the number of effector cells calculated will be an underestimate), and second, each effector cell must not functionally engage more than one target during incubation in the Ca^{2+} -free medium (or else the calculated value would be an overestimate of the effector cell incidence).

The first of the above assumptions seems warranted, for preincubation times between 30–120 min lead to the same amount of lysis following the addition of cytochalasin A and Ca^{2+} (Table 2). Indeed, in another experiment (not shown), there was no difference in the subsequent ^{51}Cr release whether cytochalasin A was added after 5 or 120 min. In all cases, however, cytochalasin A added at the same time as the target cells completely inhibited cytolysis. It would thus seem, in keeping with previous observations^{8,9,11}, that functional engagements between lymphocyte and target cell occur rapidly, and, in the conditions used in this study, are complete within 30 min.

The same arguments would seem to dictate that an effector cell does not functionally engage more than one target cell during the preincubation period. If that were the case, then extending the preincubation period would be expected to increase the number of such engagements. In fact, as earlier indicated (Table 2), preincubation periods between 30 and 120 min showed an identical number of functional interactions. It would seem unlikely that an effector cell undergoes multiple non-lytic interactions in the period 0–30 min but not thereafter, although admittedly this remains a faint possibility. It is also not likely that each effector engages several target cells simultaneously, for if this were the case, increasing the size of the target cell pool would increase the likelihood of such interactions. As Table 2 shows, however, lysis reaches a constant value not a constantly increasing one. Furthermore, extensive kinetic analysis of target cell death in ^{51}Cr -release killer assays provide evidence for "single hit" mechanisms of cytolysis⁶.

Two methods of estimating effector cells have been used previously. One approach has been to equate the frequency of cytolytic T cells with the number of target cells specifically lysed in the presence of a great excess of target cells^{6,10}. The possibility of the effector cells recycling (that is, that one lymphocyte kills more than one target cell) was largely ignored in those studies, and it is therefore likely that the calculated frequencies were overestimates. Indeed, Henney⁶ reported the incidence of effector cells in the system re-evaluated here as approximately 4% of splenic lymphocytes, a number 2–3 times higher than the incidence estimated in the present study.

The frequency of cytolytically-active cells has also been measured by counting those lymphocytes which adhere to

Table 3 Frequency of specific cytolytic T cells in the spleens of alloimmunised mice

Experiment	Days after immunisation	Maximum number ($\times 10^{-3}$) of target cells lysed per 5×10^5 viable cells	Frequency of effector cells (%)
1	10	11.0	2.2
2	10	9.0	1.8
3	10	7.0	1.4
4	10	5.0	1.0
5	10	3.5	0.7
6	11	8.0	1.6
7	11	6.0	1.2
8	11	4.5	0.9
9	12	4.0	0.8
10	12	4.0	0.8

The number of effector cells in each spleen cell suspension was determined using the experimental approach given in Table 2. Three spleens were pooled in each experiment.

Frequency $\frac{\text{maximum number of target cells killed}}{5 \times 10^5 \text{ spleen cells}}$

target cells^{3,8,11}. The critical assumption in this approach is that each lymphocyte which binds to a target cell is a specific effector cell. This is almost certainly not the case; indeed, both precursor¹² and memory cells¹³ have been shown to have affinity for homologous allogeneic cells, but neither of these T-cell populations are cytolytic^{13,14}. The contribution of non-cytotoxic lymphocytes to the overall frequency of target-binding cells remains to be established.

We have thus described a novel method for calculating the incidence of specific cytolytically active T cells. The procedure used here measures only killer T cells and obviates the deficiencies of previous approaches to this problem by preventing the effector cell from 'recycling'. Obviously the enumeration of cytolytically-active T cells provides only a partial quantification of the overall cell-mediated immune response. The quantitative relationship between this T-cell compartment and those producing lymphokines, memory and regulator cells remains to be evaluated.

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Enhancement of delayed hypersensitivity by depletion of suppressor T cells with cyclophosphamide in mice

THE delayed hypersensitivity (DH) reaction seems to be enhanced by treatment with cyclophosphamide and this has been discussed in the context of depression of antibody production¹⁻⁴. Askenase *et al.*⁵, however,

reported that low doses of cyclophosphamide augmented the reaction to sheep erythrocytes without affecting antibody production. Polak and Turk⁶ suggested that cyclophosphamide could break down immunological tolerance to the dinitrophenyl group, as measured by a skin reaction in guinea pigs after treatment with the drug to inhibit suppressor cells. The nature of the suppressing cells was not clear at the time. We report here that treatment of mice with cyclophosphamide also significantly affects DH reaction to methylated human serum albumin (MHSA)⁷ which induces little antibody production, and that the enhanced DH reaction is caused by damage to T rather than B cells. Independent recovery, from the damage due to cyclophosphamide, by individual subpopulations of T cells accounts for the kinetics of this phenomenon.

Table 2 Suppressive effect of transferred lymphoid cells on enhanced DH to MHSA in mice treated with cyclophosphamide

Cyclophosphamide treatment	Cells transferred	No. of animals	DH on day 12
150 mg kg ⁻¹	None	3	10.7 ± 1.9
150 mg kg ⁻¹	5 × 10 ⁷ Spleen cells	4	9.3 ± 1.5
150 mg kg ⁻¹	2 × 10 ⁷ Thymus cells	4	6.3 ± 2.5
None	None	4	5.3 ± 1.8

Male C57BL/6 mice (age 4 months) were given an intraperitoneal injection of cyclophosphamide (150 mg kg⁻¹) 4 d before sensitisation, and an intravenous transfer of spleen or thymus cells 2 d later.

Male C57BL/6 and (C57BL/6 × CBA)F₁ mice aged 4 or 6 months were used: they seem to be high DH responders⁸. Animals were sensitised by an injection, into the left footpad, of an emulsion (0.05 ml) consisting of equal amounts of MHSA solution (5 mg ml⁻¹ in saline) and Freund's complete adjuvant (3 mg of dead H₃₇R_v tubercle bacilli per ml). A challenge injection of 0.02 ml of MHSA solution (1 mg ml⁻¹) was given into the right footpad on day 12 of sensitisation, as the optimal DH response was obtained by this method. Swelling of the right footpad was measured both immediately before and 24 h after the challenge, and the difference between two measurements in a thickness of 0.1 mm was used to describe the intensity of the DH response⁹. Mice received an intraperitoneal injection of cyclophosphamide (given by Shionogi Co., Osaka, 15 or 150 mg per kg body weight) at intervals before or after sensitisation, as indicated in Tables 1-3. Spleens and thymuses were removed from normal syngeneic male mice and the cells from them were suspended in and washed three times with RPMI-1640 medium. Appropriate numbers of these cells were transferred into the retro-ocular plexus of the cyclophosphamide-treated mice.

Table 1 shows the effects of cyclophosphamide on the DH response to MHSA when the drug was given 8 d or 1 d before sensitisation. There was marked enhancement of DH

Table 1 Effect of cyclophosphamide on DH to MHSA in mice

Cyclophosphamide treatment	Interval between treatment and sensitisation	No. of animals	DH on day 12*	Thymus weight (mg)
None	—	4	5.3 ± 1.8	29 ± 4
150 mg kg ⁻¹	1	5	16.4 ± 1.2	23 ± 6 [14]†
	8	5	17.0 ± 6.3	38 ± 6 [21]†
15 mg kg ⁻¹	1	5	7.5 ± 1.5	35 ± 9 [14]†
	8	5	9.0 ± 4.2	35 ± 4 [21]†

Male C57BL/6 mice (age 4 months) were given an intraperitoneal injection of cyclophosphamide (15 or 150 mg kg⁻¹) at various intervals before sensitisation.

*Each value represents mean ± s.e. (0.1 mm).

†Interval between treatment with cyclophosphamide and measurement.

Table 3 Effect of cyclophosphamide on effector cells in DH to MHSa in mice

Cyclophosphamide treatment	Interval between sensitisation and treatment	No. of animals	DH on day 12	Thymus weight (mg)
None	—	3	15.2 ± 2.9	32 ± 8
150 mg kg ⁻¹	4	4	2.8 ± 0.9	14 ± 2 [9]*
	9	4	1.0 ± 0.4	14 ± 2 [4]*

Male (C57BL/6 × CBA)F₁ mice (age 6 months) were given an intraperitoneal injection of cyclophosphamide (150 mg kg⁻¹) 4 or 9 d after sensitisation.

*Interval between treatment with cyclophosphamide and measurement.

response in animals treated with the large dose. Table 2 shows that transfer of 2×10^7 thymus cells from untreated mice almost eliminated the DH enhancing effect. Transplantation of 5×10^7 normal spleen cells did not influence the enhanced DH significantly. These observations suggest that the DH to MHSa in mice is enhanced because cyclophosphamide damages suppressive T cells in the immune regulatory systems.

Table 3 shows that administration of cyclophosphamide after sensitisation suppresses the DH response. A minimal response appeared only in the group treated with cyclophosphamide on day 4 after sensitisation. These results indicate that effector T cells which are responsible for DH are also sensitive to cyclophosphamide. There have been several reports of the inhibitory effect of the drug on DH responses¹⁰, and Kerckhaert *et al.*¹¹ demonstrated the sensitivity of effector T cells. In experiments not described in Tables 1–3 we observed that previous treatment of mice with cyclophosphamide was not necessary for DH enhancement, and that treatment after sensitisation (such as in Table 3) could enhance DH only if the interval between treatment and challenge injection was sufficiently long, for example 12 d. We further observed that when the interval was just 9 d, DH was neither suppressed nor enhanced. This result together with our previous finding¹² that significant DH to MHSa was obtained as early as 4 d after sensitisation, suggest that the effector T cells, once affected by cyclophosphamide, recover sufficiently to generate significant DH response after about 5 d. This seems to be consistent with previous observations^{2,11}. The 13-d or 20-d interval between treatment with cyclophosphamide and challenge injection (Table 1) might be long enough for the effector cells recovering from the damage to be sensitised with the antigen retained at the site of injection. This may explain the apparent resistance of effector T cells to cyclophosphamide in this and some previous experiments⁶.

Thymus weight did not recover significantly until 9 d after treatment (Table 3), and then recovered considerably on day 14 and rebounded on day 21 (150 mg kg⁻¹, Table 1). Recovered lymphoid cells were histologically detectable in the cortex on day 9 after treatment, but were hardly detectable on day 4. This seems to be consistent with other observations^{10,13}, and probably supports the suggested recovery of effector T cells. But the sites recovering cells remain unknown.

The recovery of suppressor T cells is probably delayed more than that of effector cells, and seems to be within 4 weeks (unpublished observations). In previous experiments¹⁴ concerned with suppressor T cells, we found that thymus cells transferred into old recipients, which showed a maximal DH response, suppressed the development of DH to MHSa. When transfer was into young recipients who developed moderate DH to MHSa, suppression of DH was not observed. Studies on the influence of whole-body irradiation⁸ or adult thymectomy¹⁴ confirmed this, and suppressor T cells seemed to be in the category of short lived T cells. Because of a greater delay in recovery of

suppressor T cells than of effector T cells after cyclophosphamide-induced damage, the regulatory activity of the suppressors on the effectors could be minimised, resulting in an increased DH response.

Cyclophosphamide has generally been considered to have a selective suppressive effect on the B-cell functions in some circumstances. This is supported by various phenomena including suppression of antibody production¹, proportional decrease in non- θ -bearing lymphocytes¹⁵ and cell depletion in the non-thymus-dependent areas of lymphoid tissue^{13,16}, and so on¹⁰. Suppression of antibody production is often accompanied by an increase in DH response. This may indicate regulation of DH by antibodies^{2,3}.

In contrast, we conclude that T cells as well as B cells are remarkably sensitive to cyclophosphamide and therefore both DH and antibody production are defective immediately after treatment. Time for recovery from the damage is different between T cell subpopulations, that is, long-lived effector cells and short-lived suppressor or regulatory cells. The former seem to recover more promptly than the latter. We suggest that this causes enhanced DH to MHSa in mice treated with cyclophosphamide. But the possibility remains that the suppression of DH by cyclophosphamide after sensitisation is due, at least in part, to damage to bone marrow-derived monocytes rather than, or in addition to, that of sensitised effector T cells.

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reviews

Superpowers and the environment

Eric Ashby

The Economic Superpowers and the Environment: the United States, the Soviet Union and Japan. By Donald R. Kelley, Kenneth R. Stunkel and Richard R. Wescott. Pp. viii+335. (Freeman: San Francisco, 1976.) Hardcover \$11.95; softcover \$5.95.

A BOOK on this topic was badly needed. The authors have met the need admirably. They modestly point out that their book is not a comparative study of environmental policies in the three superpowers, but is rather three profiles, one for each of the three nations they have chosen. They urge the reader to make his own comparisons and they make this easy for him by the arrangement of the book; for instead of dividing it into three sections, one for each country, they have divided the book into topics—the different kinds of environmental hazard, the attitude of the public toward the problems, the politics of environmental quality, and programs for the protection of the environment. Under each of these topics they discuss the recent history and the contemporary position in the US, the Soviet Union, and Japan.

Any reader familiar with the gush of doomsday literature in the past five years may be a little uneasy that the authors make some of this the starting point for their story, without reference to the criticism which has devalued some of this literature. But any unease is dispelled once the authors get down to the job they have set themselves. Their own exposition is clear, well documented, and on the whole free from those emotive adjectives which diminish the credibility of much writing about the environment. A feature which makes the book unusually interesting is the way the authors have put environmental problems into their historical and cultural and economic and political settings.

The first chapter describes an America, a Soviet Union, and a Japan which respected nature: the pastoral landscape celebrated by Jefferson, peopled by prudent farmers, conserving their heritage; the Slavophile closeness to nature, preached by Tolstoy; the exquisite harmony between man and nature in the Japan of Tokugawa. The book then goes on to give factual data about the degree of pollution (air,

water, solid wastes, radioactivity, noise) in the three countries. Here they are up against the coyness of the Soviet Union in disclosing data comparable to that which the American Council on Environmental Quality issues each year; but they do present a remarkably useful set of facts, all the same.

It is a grim picture: neglect, in spite of the evident dangers in Japan (by far the most gravely injured country in consequence of its industrial development); indifference, due to the immense size of the Soviet Union (where the only measure of production which earned political approval was *quantity*, without regard to its second order effects); and frustration, in spite of the spate of legislation in the US and the vast sums being spent on environmental protection. All these countries face environmental problems which sooner or later are likely to lead to major social crises, best described as an obligation to change their cherished styles of life.

Japan is closest to the crisis, but her dilemma may be redeemed by the age-old tradition of "group co-operation, self-discipline" and the obedience which comes of "traditional subservience of nearly everyone to somebody else." She has changed her life style twice in the last century; she might be capable of doing it again.

America is (in the authors' opinion) going through a cyclical pattern of waxing and waning concern for the environment. When the National Environmental Policy Act was passed, cleaning up pollution came high in the public opinion polls; but the public assumed that once legislation was

passed, all would be well, and the oil crisis swiftly eclipsed interest in protecting the environment; it left no doubt that Americans put a higher price tag on gasoline for their cars than on cleaner air in their cities.

The Soviet Union has the best chance of anticipating the dangers of exploiting the environment, because she is not so far along the road of exploitation. Moreover she does not need to woo public opinion nor to tame corporations in order to adapt their life style to a pattern which takes better care of the environment.

This sober, level-headed book concedes that Japan and the Soviet Union, with highly centralised government and traditions of obedience to authority, could more easily extricate themselves from the ecological trap into which industrialisation leads. But they end with a hopeful paradox: that the open society in the US, where public opinion counts for much more, may in the end be better equipped to sustain a long term adaptation to a world with too many people, too few resources, and limitations to the capacity of air and water to contain our wastes. Hanging over the story there is Heilbroner's threat: "who cares, in the perspective of environmental safety, if institutions of present-day capitalism and socialism disappear?"

Eric Ashby (Lord Ashby of Brandon) recently retired from the Mastership of Clare College, Cambridge. He was Chairman from 1970 to 1973 of the UK Royal Commission on Environmental Pollution.

Insight marred by error

The Biology of Population Growth. (Biology and Environment.) Robert L. Snyder. Pp. 227. (Croom Helm: London, April 1976.) £6.95.

THIS broadly-based account of population biology lays special emphasis on mammalian studies, with an American slant—Dr Snyder is director of a research laboratory at Philadelphia Zoo. Chapters deal with Ecology and Population, Reproduction, Mortality, Dispersion and Dispersal, Population Dynamics, Natural

Regulation, Compensatory Mechanisms, and Implications to Human Populations. The treatment is comprehensive in the sense that it touches at least briefly on most aspects of the subject. Some are dealt with in more detail, including the population cycles and reproductive physiology of mammals, the social stress theory of population regulation, experiments on the social organisation of captive mouse colonies, and Dr Snyder's own study of compensatory processes in populations of woodchucks. On this

last topic, the account seems to be too detailed and too much like a research paper, but it is the concentration points that give the book an individuality and special interest. There is an eight-page index.

In a work on this subject, algebraic statements on population dynamics play an essential part. I noted seven such equations that were wrongly printed (on pp136 (two), 137 (two), 162, 164 and 173)—pitfalls, ranging from obvious to deadly, for the unwary student. Too many small verbal errors crop up in the text, but these are not likely to mislead. Here and there inaccurate or oversimplified statements occur: although variation is adequately dealt with elsewhere, on p17 populations are called assemblages of organisms with the same heredity, and the idea is repeated on p23. Does biomass include biovolume (p27)? The surplus members of a population are wrongly referred to as standing crop (p30); p49 states that in protandry one gonad alternately produces eggs and spermatozoa; fecundity is equated with sexual maturity (p92). It seems unhelpful to have movements (within the home range) included in dispersion (pp141 and 148). Contrary to what is stated on p159, the straight part of the curve in Fig. 18 does not indicate exponential growth in numbers, and the calculation of the rate of increase is inappropriate.

It is a pity that a book which gives an excellent insight into population biology should be marred in these ways. Experienced and critical students of the subject are likely to find it valuable, but I would hesitate to recommend it for undergraduates until it is carefully revised.

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Donor-acceptor complexes

Donor-Acceptor Bond. By E. N. Guryanova, I. P. Goldstein and I. P. Romm. Pp. ix+366. (Wiley: New York and Chichester; Israel Program for Scientific Translations: Jerusalem, December 1975.) \$39.60; £19.80.

A VERY large number of books on electron donor-acceptor (or charge-transfer) complexes is now available. Many of these books are quite excellent. Since any new book on this topic must be judged in terms of its predecessors, its acceptance is very much an up-hill affair.

The craftsmanship of the book, quite apart from binding and paper, is poor. Page and text edges are not parallel, margins are of variable width, and the English is simply bad. For one sensitive to language, this

book is a painful business. This, of course, is the fault of the translation service, not of the authors.

Chapter I, "Theoretical Principles of The Electron Donor-Acceptor (EDA) Interaction", contains the gist of the present status of EDA concepts. Although complete, it is not incisive and it leaves behind no great residue of understanding.

The novelty of the book is mostly contained in Chapter II, "Methods for Studying Molecular Compounds". This chapter is the most complete discussion of the determination of thermodynamic data for complexes which this reviewer has seen. The discussion treats such obscure (but obviously useful) methods as dielectrometry in a most informative way. I found this chapter to be of pedagogic use.

Chapter III, "Physicochemical Properties of EDA Complexes", is an excellent discussion of the properties, mostly thermodynamic, of EDA complexes. Its most distinctive feature is its immense set of Tables—128 pages of them (926 refs). The volume of data contained in these tables—all of which are confined to the ground state but cover almost all physically measurable properties—is massive. It is these tables which make library purchase of this book mandatory.

Chapter IV, "Complexes of the Halogens and Halides of Elements with Aromatic Hydrocarbons", probably represents one of the authors' (that is Romm's) interests and may well be the reason why such an ungeneral topic merits one out of five chapters. In any event, the chapter is informative and well written.

Chapter V, "Some General Characteristics of Donor-Acceptor Interaction", like all terminal chapters, is an accretion of all those important things which should have been said elsewhere, as well as a summary of general properties, mostly correlative, of EDA complexes.

In sum, the book survives the acid tests. It is a book of which its authors can be proud. I recommend purchase by libraries and by individuals directly involved in this research area.

S. P. McGlynn

Lysosomes

Lysosomes: A Survey. (Cell Biology Monographs.) By E. Holtzman. Pp. xi+298. (Springer: Wien and New York, 1976.) DM130.

INFORMATION about lysosomes is widely scattered in journals concerned with biochemistry, cell biology, ultrastructure, pathology, microbiology and immunology, and it is useful to have available information put

together for reference. The standard reference work is the multi-volume *Lysosomes in Biology and Pathology*, which has acquired a somewhat biblical status. For personal reference and teaching purposes this monograph by Holtzman will in most cases be enough. It is clearly written, abundantly and well illustrated (the author is a cell biologist whose specialty is electron microscopy) and quite comprehensive.

Indeed, the text is occasionally discursive. Space is given to hydrolases in bacteria and their role in protein turnover and cell wall degradation. Even though these hydrolytic enzymes are not confined within lysosomes, the analogy with lysosomes is interesting. As expected, Holtzman is strongest on morphology; and the interrelations of phagocytic vacuoles, multivesicular bodies, cup-like bodies, the Golgi apparatus, secretion granules, autophagosomes and residual bodies are described with the relish which a captain takes in guiding his boat among reefs and offshore islands.

Nevertheless, Holtzman has a clear understanding of the biochemical and biophysical aspects of lysosomology, and the sections on intracellular digestion and permeability of lysosomal membranes to the products systematically cover available information. In the section on storage diseases Brady's helpful list of missing enzymes and striking electron micrographs of the storage vacuoles in neurones and other cells are presented.

Each reviewer will find sections of the book with which he disagrees. I was least satisfied with the sections on lysosomes in cytotoxicity and enzyme secretion. For example, macrophages that have ingested silica particles are rapidly killed. The particles are clearly in secondary lysosomes soon after ingestion, so that it can scarcely be denied that the cytotoxic effects begin in the secondary lysosomes. Dispute about possible artefacts of histochemistry are irrelevant. The section on extracellular release of lysosomal enzymes is a list of papers of uneven quality, obscuring in a mass of marginally relevant detail the important implications of the phenomenon in inflammation. But what seems obvious to one investigator may be less convincingly demonstrated to another, and Holtzman's presentation is, in general, critical and well balanced. It should serve as an introduction to the new generation of lysosomologists who will clear up many of the uncertainties left by the first generation.

A. C. Allison

Premature perspectives

Psycholinguistics: Introductory Perspectives. By Joseph F. Kess. Pp. xii+268. (Academic: New York and London, March 1976.) \$9.95; £5.50.

CHOMSKY has revolutionised linguistics by proving the inadequacy of certain informal theories of language and establishing a formal theory of his own. His work made an early impact on the budding science of psycholinguistics, which blossomed in the attempt to establish the relevance of transformational grammar to the mental processes used in understanding and remembering sentences. By the time psychologists had discovered that such matters were more complicated than they had feared, Chomsky had moved on and reformulated his theory. That was more than ten years ago, and since then his school has splintered into a number of rival factions. Psycholinguistics is in a similarly confused state, and it is hard to imagine how anyone could have both the temerity and the clarity of vision to write a textbook on the subject. Nevertheless, a number of excellent introductions have appeared.

In searching for a niche for his book, Kess has tried to write a text that can serve as "a first set of readings with which to orient oneself in the field of language behaviour." It should be clear from the history of the subject that the task is an exacting one. Unfortunately, Kess is not equal to it. He has not mastered the literature, and his book is flawed by too numerous inaccuracies and distortions. His description of the essential characteristics of transformational grammar is wrong. And it is hard to imagine a more misleading account of the notion of a grammatical rule—in the context of a discussion of Chomsky—than the following: "a rule is simply a convenient summarisation of the observations of a normal, or statistically frequent, type of behaviour in a given situation." This definition manages to combine in a single sentence more heresies to the true dogma than one would have thought possible. For Chomsky, of course, a grammatical rule characterises part of a speaker's tacit knowledge of a language. Observation of statistical regularities and induction from them does not suffice to establish a rule because there are precious few regularities to observe unless a listener is sensitive to structure; and the structure of an utterance is rarely manifest in the noises a speaker produces. It is for this reason that Chomsky argues that these are innate constraints on the sorts of rules that are considered in acquiring one's native tongue.

Kess writes in a way that is seldom clear and often misleading. Even his virtues turn out to be double-edged. His impartiality renders no school of thought immune from misrepresentation. His originality verges on idiosyncrasy—as when he suggests that linguistic theory should explain why a sentence happens to be false. His zeal for the truth leads to banality—as when he tells us that formal language is typically reserved for those social situations judged to be formal. But, enough. This book fills a much-needed gap. It is dangerous for anyone to read it who needs to read it.

P. N. Johnson-Laird

Plant and animal lectins

Receptor-Specific Proteins. By Edwin R. Gold and Peter Balding. Pp. xiii+440. (Excerpta Medica: Amsterdam; American Elsevier: New York, 1975.) Dfl.125; \$51.95.

THIS is the first of what promises to be an outpouring of monographs on lectins. The term lectin (Lat. *legere*, to pick out or choose), proposed by Boyd after he discovered that the lima bean agglutinin (phytohaemagglutinin) was blood group A specific, is currently also applied to invertebrate and even vertebrate agglutinins. The problems of lectin definition are recognised by Gold and Balding in extensive coverage in the introductory and final chapters, in which they suggest using the term receptor-specific proteins (RSP). Unfortunately, this contributes little toward clarifying the situation since the term "receptor" must then be defined. The fact that no known physiological function has been assigned to these substances has complicated the development of a systematic nomenclature. Nevertheless, lectins have been grouped according to the specific carbohydrates with which they interact and the human erythrocytes (ABO and MN systems) which they agglutinate.

The book is "arranged as far as possible according to present taxonomy". Thus, Chapter 2 deals with viruses. (The fact that some viruses interact with carbohydrate structures is not sufficient reason to include them under the definition of lectins; this reviewer believes all 'organisms' should be disqualified from consideration as lectins.) Chapter 3 covers lectins of bacterial and algal origin; and Chapter 4 treats lectins from lichens and fungi (for example, from the common meadow mushroom *Agaricus bisporus* and the mold *Streptomyces* sp.).

The largest group of lectins studied so far derives from the seeds of flower-

ing plants (*Embryobionta*), the subject of Chapter 5. A large number of plant seed lectins have been purified and their physicochemical properties investigated, principally during the past five years. A milestone in lectin research was reached two years ago with the elucidation of the primary and X-ray crystallographic structure of concanavalin A—the jack bean lectin, first studied by Sumner and Howell over 40 years ago.

Chapters 6–9 deal with lectins found in protozoa, sponges, molluscs (especially clams and snails), arthropods (horseshoe crab and lobster), and lower vertebrates (fish roe seemingly a very good source of agglutinins). The chapters on invertebrate lectins are especially well done.

The enormously renewed interest in lectins (first discovered in 1888 by Stillmark investigating extracts of the castor bean, *Ricinus communis*), results from their remarkable biological properties. In addition to their roles as haemagglutinins, reagents in blood group serology, and structural probes for investigating the nature and distribution of cell-surface carbohydrates, lectins have been shown to act as mitogens, serve as agents for studying membrane fluidity, and function as reagents for distinguishing between malignant and normal cells. Chemically linked to insoluble supports, lectins have been used to isolate enzymes, hormones, a variety of plasma proteins, and cell-surface glycoproteins.

Writing a monograph on lectins at this stage in the explosive development of the field was an ambitious (if not impossible) undertaking. Although the authors present good historical statements on both plant and animal agglutinins, the treatment of plant lectins is abbreviated and somewhat disappointing. The authors recognise this in their qualification that plant lectins deserve full and complete treatment in a separate monograph. At the time of printing, however, there was considerable information available on wheat germ agglutinin and the lentil lectin, for example, which could have been included in the discussion. Even with an addendum to each chapter, the volume is already out of date due to the enormous activity in the field. A good general and generic index plus tables at the end of each chapter are valuable contributions to the volume enabling rapid reference to an enormous number of lectins. Apart from several reviews, however, this is the first monograph to be published with relatively broad coverage of both plant and animal lectins; as such it deserves serious attention from all those interested in 'lectinology'.

Irwin J. Goldstein

Spectral and Chemical Characterisation of Organic Compounds: A Laboratory Handbook. By W. J. Criddle and G. P. Ellis. Pp. viii+103. (Wiley: London and New York, February 1976.) £4.60; \$10.15.

THE avowed purpose of this book is to bring together, in one small volume, the information a student is likely to need in the laboratory to enable him to establish the identity of organic compounds whose structures are unknown to him. The chemical part of the book is of a familiar type embracing short chapters on preliminary tests, separation of mixtures, the preparation of derivatives, followed by 50 pages of tables of melting points of these derivatives. There is also a short chapter on pharmaceutical compounds.

The book's main claim resides in the second chapter—chemical and spectroscopic characterisation of functional groups—and it must be said that there is not really sufficient emphasis on spectroscopic data; there is, for example, no mention of mass spectra at all. Students will thus need to buy a book on the interpretation of spectra; they are unlikely to buy this book as well just for its melting point data—which they can obtain from the larger reference books in the laboratory in any case.

Peter Sykes

Mosquito Ecology: Field Sampling Methods. By M. W. Service. Pp. xii+583. (Applied Science: London, 1976.) £30.

ALMOST as many ways have been devised of collecting mosquitoes as there have been field workers. In his book Dr Service has reviewed some hundreds of these methods, and managed to do so in a readable and informative fashion. Many of the traps and sampling devices were designed simply for the collection of large numbers of individuals, but others provide data which contribute towards an understanding of mosquito ecology. The latter are rightly given more emphasis, with accompanying descriptions of methods of analysing the data.

The early chapters deal with the sampling of eggs and larvae, but the bulk of the book is concerned with adults. Descriptions are given of traps for adults as they emerge from their larval habitats and for sampling from resting sites. Attractant and non-attractant traps are most fully covered, and a short chapter is included on the use of experimental

huts for evaluating insecticides. The descriptions are supplemented with clear line drawings.

The sections dealing with population size estimation—for example, by mark-release-recapture techniques or sequential sampling—and the chapter on estimating mortality in immatures and adults are perhaps too condensed, but there is compensation in the full bibliography.

The book was written for the field worker; it should meet his needs admirably.

W. W. Macdonald

Books brief

The Brown Rat. By Graham Twigg. Pp. 150+8 plates. (David and Charles: Newton Abbot, London, North Pomfret, Vermont and Vancouver, January 1976.) £4.95.

THIS chatty account of *Rattus norvegicus* is evidently addressed primarily to laymen. It has, however, a useful, if patchy, bibliography, and some good information on the brown rat as a food pest and vector of disease. Topics include populations and other aspects of rat ecology, reproduction and—too briefly—control. Examples are mainly from Britain and North America. Among diseases, special attention is given to plague and leptospirosis. The account of the biology of rats does not do justice to their very important exploratory behaviour. The author is also quite at sea on their social conduct. There are plenty of illustrations—some good—and an index. The book may serve to introduce readers to aspects of economic zoology and public health that ought to be more widely known.

S. A. Barnett

Membranes: A Series of Advances. Vol. 3, Lipid Bilayers and Biological Membranes: Dynamic Properties. Edited by George Eisenman. Pp. xiv+538. (Dekker: New York and Basel, 1975.) \$42.50.

THE proliferation of books has rarely little to commend it, but Volume 3 of Eisenman's *Membranes* is quite definitely an exception. It brings together in one volume a group of first-class papers by acknowledged

experts in the field of ion permeation and selectivity in biological systems which will be of great value to everyone—student, teacher and research worker—interested in this field.

Chapters on the Structure and Dynamic Properties of Ion-Specific Antibiotics (Grell, Funck and Eggers), The Kinetics of Carrier-Mediated Ion Permeation in Lipid Bilayers and its Theoretical Interpretation (Laprade, Ciani, Eisenman and Szabo) and The Action of Uncouplers on Lipid Bilayer Membranes (Neumcke and Bamberg) are set alongside chapters on Ionic Selectivity of Na and K Channels of Nerve Membranes (Hille), Potassium Pores of Nerve and Muscle Membranes (Armstrong), Ca-Dependent Action Potential (Hagiwara) and Cation Permeation Mechanisms and Cation Selectivity in Tight Junctions of Gallbladder Epithelium (Moreno and Diamond).

This book is well produced and readable and should remain a valuable reference work for a number of years.

P. F. Baker

Laser Doppler Measurements. By B. M. Watrasiewicz and M. J. Rudd. Pp. viii+160. (Butterworths: London and Boston, Massachusetts, 1976.) £6.90.

THIS is an attractive little volume in which the principles and the practice of laser anemometry—the measurement of the velocity of winds and related phenomena—are outlined in stages of progressive complexity. Out of the wealth of dynamic phenomena which can be investigated by study of the light scattered from particles in motion, the authors have chosen to concentrate on the single topic of laminar and turbulent fluid flow. They discuss in succession historical developments, principles of the laser and scattered light detection, simplified theories of laser anemometers, as well as more detailed aspects; problems related to signal processing in the frequency and time domains, measurement of turbulence parameters and applications in wind tunnels and in the atmosphere. Being myself a non-specialist in anemometry but deeply interested in other aspects of laser light scattering, I found the description and analysis rewarding. I believe the book to be equally useful to novices of this discipline. It would seem, however, that the specialist working in the field is well acquainted with the bulk of the material.

H. Eisenberg